

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.

For more information visit www.intechopen.com



Hydrogels for Regenerative Medicine

Divya Bhatnagar, Marcia Simon and Miriam H. Rafailovich

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/62044>

Abstract

Regenerative medicine requires materials that are biodegradable, biocompatible, structurally and chemically stable, and that can mimic the properties of the native extracellular matrix (ECM). Hydrogels are hydrophilic three-dimensional networks that have long received attention in the field of regenerative medicine due to their unique properties. Hydrogels have a potential to be the future of regenerative medicine due to their desirable mechanical and chemical properties, ease of their synthesis, and their multiple applicability as drug delivery vehicles, scaffolds, and constructs for cell culture. In this chapter, we have described hydrogels in terms of their cross-linking and then discussed the most recent developments in the use of hydrogels for peripheral nerve regeneration, tooth regeneration, and 3D bioprinting.

Keywords: Hydrogels, nerve regeneration, 3-D printing, tooth regeneration

1. Introduction

Two-dimensional (2D) substrates such as tissue culture polystyrene (TCPS), thin films, and other flat surfaces have traditionally been used to culture mammalian cells *in vitro*. These experiments with the 2D cell constructs have not only provided the basis for understanding complex biological processes but have also led the way in exploring the stem cell differentiation, cell–material interactions, and cell–cell interactions [1]. Three-dimensional (3D) scaffolds were designed to mimic the important physiochemical features of the native cellular microenvironment for *in vitro* cell culture. Among these 3D scaffolds, hydrogels are defined as the cross-linked polymer networks with high water content. Hydrogels are viscoelastic in nature, encompassing both the viscous and the elastic properties. They swell strongly in aqueous media and are typically composed of a hydrophilic organic polymer component that is cross-linked into a network by either covalent or noncovalent interactions [2, 3]. Cross-linking provides structural stability, and the high water content provides fluid-like transport proper-

ties [4]. Variation in the cross-linking of these hydrogels also allows for tunable mechanical properties which can be used to evaluate the structure–function relationship at the cell/biomaterial surface. Currently, hydrogels used for mammalian cell culture are synthesized from natural and synthetic materials. Bioactive hydrogel constructs are extensively being used to repair, regenerate, or engineer tissues by being able to promote cell adhesion, migration, proliferation, and stem cell differentiation appropriate to particular tissues [5]. To fully understand the cell functional responses in the context of a particular tissue, recently, many researchers have tried to develop physiologically relevant, biocompatible, biodegradable hydrogel constructs that resemble native tissue and very closely mimic the actual *in vivo* conditions [5–7].

In this chapter, we will review the classification of natural and synthetic polymer-based hydrogels in terms of their cross-linking. Recent advances in the application of novel hydrogels for regenerative medicine areas such as their use in peripheral nerve regeneration, tooth regeneration, and 3-D printed scaffolds would also be addressed.

2. Classification of hydrogels

One way of classifying the hydrogels is through the type of cross-linking [8]. Cross-linking maintains the hydrogel network structure and prevents dissolution of the hydrophilic chains.

2.1. Physically cross-linked hydrogels

Physically cross-linked gels, also known as reversible gels, are networks that are held together by attractive noncovalent forces between the polymer chains (Figure 1). These hydrogels have a tendency of going through a transition from a three-dimensional stable state to eventually degrade and dissolve as a polymer solution. These forces that hold these polymer networks together to form a hydrogel, which includes hydrophobic interactions, hydrogen bonding, or ionic interactions [9, 10].

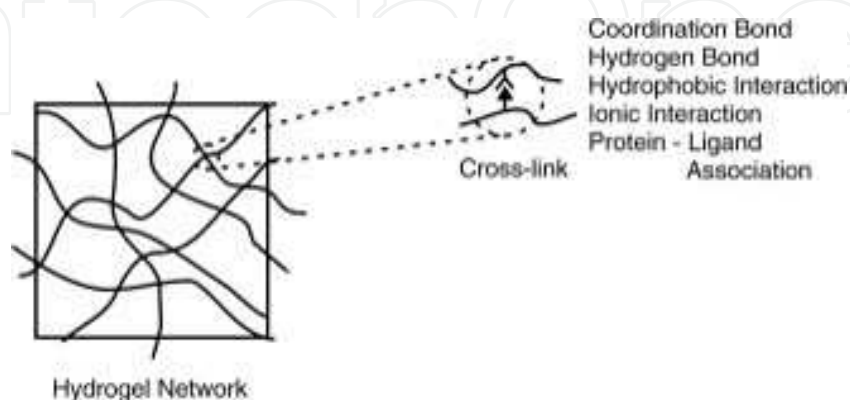


Figure 1. Physical cross-linking in hydrogels, in which the cross-links are formed via noncovalent interaction. Reproduced from ref. [11] © John Wiley and Sons.

Physically cross-linked hydrogels have found their use as matrices for cells/drug encapsulation and release, as scaffolds for cell growth, proliferation, and adhesion. Collagen, gelatin, hyaluronic acid (HA), and alginate are the most commonly used natural polymers, which form physical hydrogels. However, these physically cross-linked hydrogels are prone to premature degradation by proteolytic enzymes such as gelatinase for gelatin, collagenases for collagen, and hyaluronidase for HA [12]. On the other hand, physically cross-linked gels such as pure non-modified HA exhibits poor biomechanical properties [13] and gelatin dissolves into a solution at higher temperatures. Many researchers have therefore tried to formulate physically cross-linked hydrogels with improved mechanical properties and better cell adhesion properties. For example, a composite hydrogel of HA and gelatin was formulated by intercalating the polymer chains into laponite clay by ion exchange. The resulting hydrogel had improved mechanical properties and cell-adhesive surface [7, 14]. Another example of cross-linking by ionic interactions is that of dextran, which forms a hydrogel in the presence of potassium ions [15]. Alginate, a polysaccharide, can also be cross-linked with divalent calcium ions to form a hydrogel [8].

Synthetic polymers such as the triblock copolymer poly(ethylene oxide)₉₉–poly(propylene oxide)₆₇–poly(ethylene oxide)₉₉ (PEO₉₉-PPO₆₇-PEO₉₉, Pluronic F127) can also form a physical hydrogel via hydrogen bonding. Pluronic F127 is unique for its hydrophobic interactions between triblock copolymer chains. At low temperatures, both PPO and PEO chains are soluble in water. Above the critical solution temperature (CST) at which gelation occurs, the polymers dissolve due to the breaking of hydrogen bonds between water molecules and the chains, and PPO becomes hydrophobic PPO core and PEO corona, forming a face-centered cubic nanostructured hydrogel. At even higher temperatures, the micelles aggregate together into hexagonally packed cylinders [16, 17]. Blends and interpenetrating networks of two dissimilar polymers can also form physical hydrogels through noncovalent cross-links. The pure F-127 hydrogel has reduced mechanical properties and, therefore, it has been blended with HA and gelatin to improve its mechanical properties [6]. Other synthetic polymers such as poly(acrylic acid) and poly(methacrylic acid) form physical hydrogels by forming hydrogen bonds with poly(ethylene glycol). This kind of hydrogel formation is pH-dependent since the hydrogen bonds are formed only when the acid groups are protonated [18, 19].

2.2. Chemically cross-linked hydrogels

Chemically cross-linked hydrogels, also known as “permanent” gels, were cross-linked networks formed due to covalent bonds. These gels are usually more stable than the physically cross-linked hydrogels and have a permanent structure [8, 20, 21]. Polymerizing monomers in the presence of cross-linking agents typically forms chemically cross-linked gels. Poly(2-hydroxyethyl methacrylate) is a well-known hydrogel-forming polymer which is generally synthesized by radical polymerization of HEMA in the presence of a suitable cross-linking agent (e.g., ethylene glycol dimethacrylate) [8]. Figure 2 shows a schematic example of the formation of a chemically cross-linked hydrogel via radical polymerization. Hydrogels can also be formed by cross-linking of the various functional groups present in the polymer backbone. Polymers containing hydroxy, amine, or hydrazide groups can be cross-linked by

using glutaraldehyde, which forms covalent bonds with each of these functionalities [4]. The swelling, mechanical strength, elastic modulus, diffusional, and other physical properties of these chemical hydrogels are mainly dependent upon their degree of cross-linking, method of preparation, polymer volume fraction, temperature, and swelling agent [22].

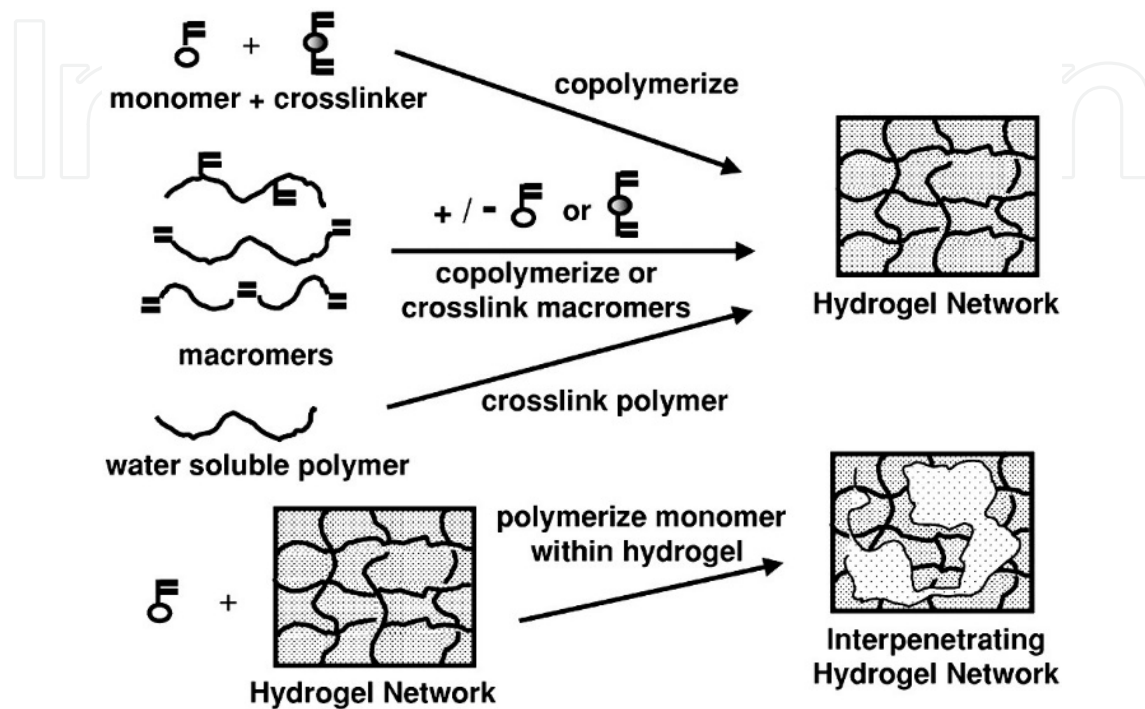


Figure 2. Schematic of methods for formation of cross-linked hydrogels by free radical reactions, including a variety of polymerizations and cross-linking of water-soluble polymers. Examples include cross-linked PHEMA and PEG hydrogels. Reproduced with permission from ref. [2] © Elsevier.

Covalently cross-linked hydrogels can also be formed via enzymatic cross-linking. For example, gelatin, which is chemically cross-linked using glutaraldehyde and formaldehyde to form a stable hydrogel, can also be cross-linked with microbial transglutaminase (mTG) to form an enzymatically cross-linked system. Transglutaminases are a class of natural enzymes that catalyze the acyl-transfer reaction between the ϵ -amino group of lysine and the γ -carboxyamide group of glutamine in proteins [23, 24]. Microbial transglutaminase (mTG) catalyzes the formation of N- ϵ -(γ -glutamyl) lysine amide bonds between individual gelatin strands to form a permanent network of cross-linked gelatin [25]. This permanent network of gelatin offers multiple focal adhesion sites for cell attachment, proliferation, and migration.

Another class of hydrogels is the stimuli-responsive hydrogels. These hydrogels can show significant changes in their swelling behavior owing to subtle changes in the pH, temperature, electric-magnetic field, and light [21]. The behavior of these stimuli-sensitive hydrogels depends on the type of the polymer used in making the gel and/or any post-polymerization modifications that are made [26, 27]. pH-responsive hydrogels are swollen ionic networks containing either acidic or basic pendant groups which in an aqueous environment of appropriate pH, ionize developing fixed charges on the gel and thus increasing the swelling forces

[22]. The use of stimuli-sensitive polymers in fabricating hydrogels has led to many interesting applications. Poly(N-isopropylacrylamide) (pNIPAm) is the most widely studied stimuli-responsive polymer. It is formed from the monomer N-isopropylacrylamide ($\text{H}_2\text{C}=\text{CHCONHCH}(\text{CH}_3)_2$) that exhibits temperature-sensitive swelling behavior over a temperature range of interest. pNIPAm has a lower critical solution temperature (LCST), below which the polymer is soluble. This is attributed to its coil-to-globule transition [28, 29]. Researchers have shown that it is possible to form a strong, thermally responsive nanocomposite hydrogel within a physiological temperature range by initiating free radical polymerization of NIPA from the clay surface [30–32]. Unique properties of cross-linked nanocomposite PNIPA hydrogels has enabled its use as drug delivery systems, rapid release cell culture substrates (Figure 3), and as wound healing dressings.

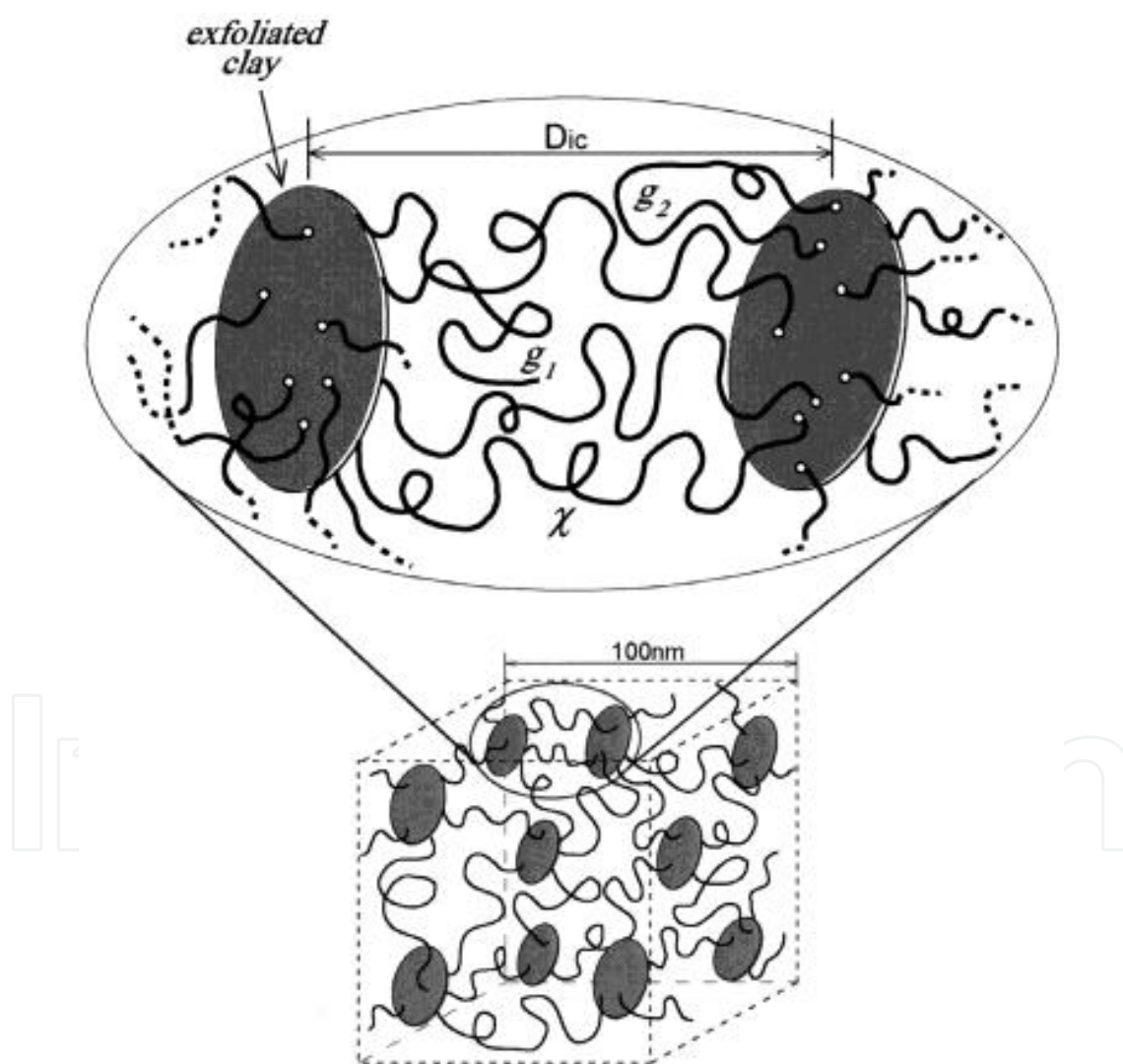


Figure 3. Schematic representation of the structural model with organic/inorganic networks in the NC gel. D_{ic} is an interparticle distance of exfoliated clay sheets. θ , g_1 , and g_2 represent cross-linked chain, grafted chain, and looped chain. In the model, only a small number of polymer chains are depicted for simplicity. Reprinted (adapted) with permission from ref. [33] Copyright (2003) American Chemical Society

3. Application of hydrogels in regenerative field

Field of regenerative medicine works with a common goal of repairing and regenerating damaged tissues and organs. The regenerative process encompasses isolating living cells from patients, expanding them *in vitro* using polymeric scaffolds, and then to re-implanting the tissue-like constructs into the patient [34]. Because of their versatile properties, hydrogels have found several applications in the field of regenerative medicine as scaffolds for cell culture and delivery vehicles for cells and genes [35]. These hydrogels can be made biocompatible with tunable mechanical and degradation properties. They can be equipped with biological cues to guide adhesion, migration, and proliferation of cells and binding sites for growth factors, peptides, or cytokines. This allows for the formation of biomimetic hydrogels that can mimic the extracellular matrix (ECM) environment.

3.1. Hydrogels for peripheral nerve regeneration

Peripheral nervous system (PNS) can repair itself after an injury, but this process has its limitations beyond the critical size gap. Nerve grafts are an alternative to repairing severe peripheral nerve injuries. Nerve autograft and allografts are often used for nerve injuries that cannot be repaired by direct coaptation. However, nerve autografts have several limitations including donor site morbidity, limited availability of the donor tissue, and limited functional recovery. On the other hand, allografts require the use of immunosuppressants for over 18 months and hence, have a significant drawback in their applicability [36]. Nerve guidance tubes (NGTs) fabricated from natural or synthetic biomaterials, for this reason, have become an attractive alternative to repairing critical size nerve defects. NGTs act as a connecting bridge between the proximal and distal ends of the severed nerve, where the nerve stumps are inserted into the ends of the tube and sutured together. A protein-rich fluid containing growth-promoting substances is released into the NGTs. Within days, a fibrin cable is formed that supports the migration of Schwann cells (SCs) and facilitates axonal regeneration from the proximal to the distal stump (Figure 4) [37].

Hydrogels as conduit material: Collagen is an important extracellular matrix (ECM) component that has been studied quite extensively in peripheral nerve regeneration. Collagen hydrogels have been used successfully for the *in-vitro* culture of many neuronal cell types. Many researchers have developed collagen-based nerve conduits to repair short nerve gaps [38]. Few examples of commercially available, FDA-approved collagen-based tubes that have been clinically used are NeuroGen, NeuroFlex, NeuroMatrix, NeuroWrap, and NeuroMend [39–43]. A nerve tube fabricated from highly purified type I + III collagen derived from porcine skin, Revolnev, has also been used to repair 1 cm rat peroneal nerve with satisfactory functional recovery [44]. Aligned collagen conduits developed by Phillips et al. were shown to orient SCs *in vitro* and their implantation *in vivo* resulted in higher axonal regeneration in a rat sciatic nerve injury model [45]. Hyaluronic acid, a naturally occurring polysaccharide, is another ECM component that has been used to fabricate nerve conduits in a modified form. Sakai et al. made an HA-based nerve conduit that facilitated cellular and axonal ingrowth during peripheral nerve regeneration by identifying viability of disseminated Schwann cells and neuron cells

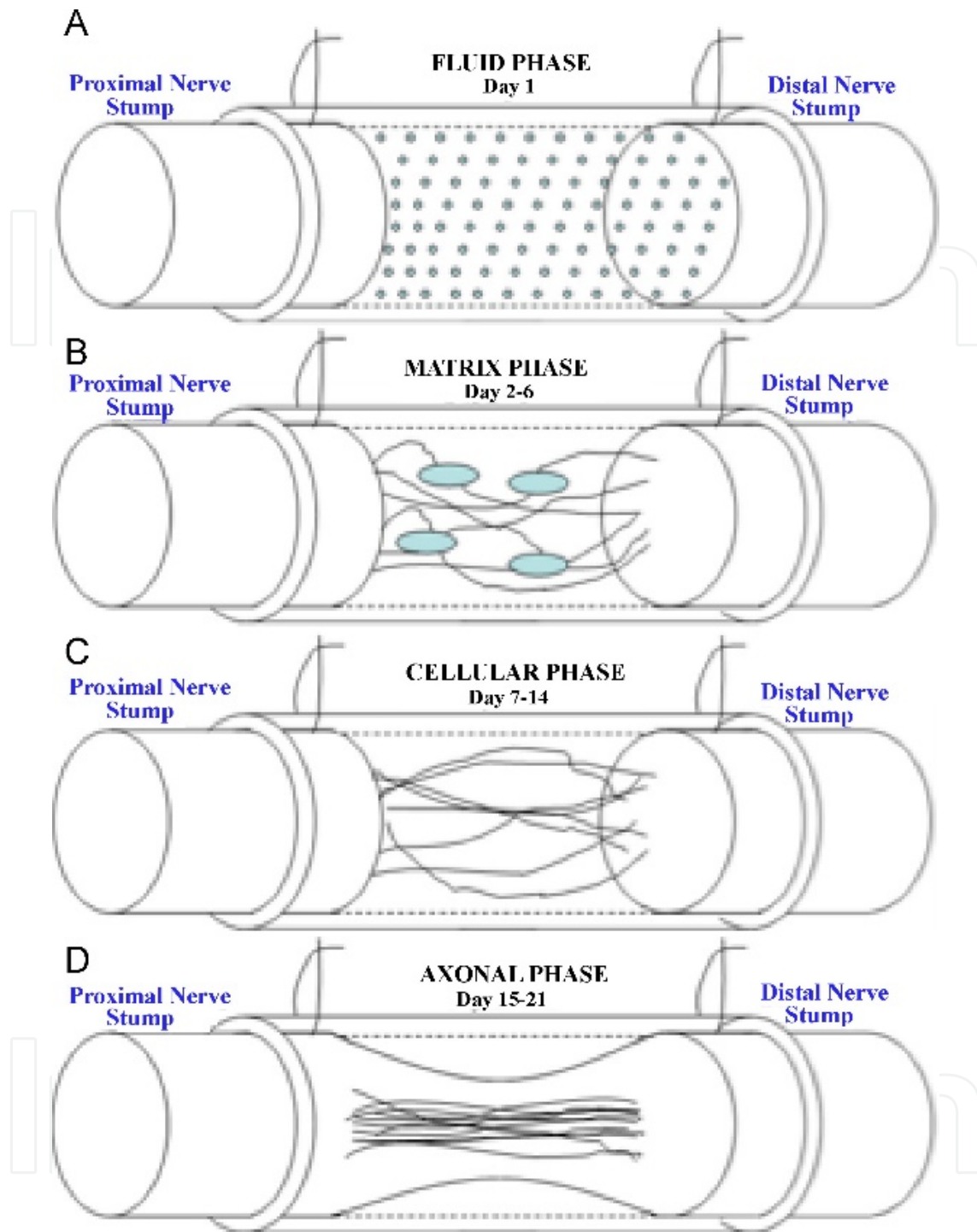


Figure 4. Principle of nerve entubulization and the sequence of events leading to the growth of a new nerve cable: (A) chamber walls with a protein-rich fluid (containing neurotrophic factors); (B) generation of a fibrin-rich scaffold; (C) cell migration (perineural, endothelial, and Schwann cells); (D) axonal cables elongation. Reproduced with permission from ref. [37] © Elsevier.

into HA conduits *in vitro* [46]. Jansen et al. prepared nerve conduits from an esterified hyaluronan derivative (Hyaff) by individual knitting of the strands and strengthening it by coating a thin layer of the same polymer [47]. Fibrin glue/gel is an FDA-approved sealant that

contains fibrinogen and thrombin. Fibrin is a protein that is involved in normal blood clotting, while fibrin gel has been extensively used in peripheral nerve regeneration as a sealant for coaptation of nerves [48]. Fibrin gels have also been used as nerve conduit to promote nerve regeneration [49–52]. Pettersson et al. [52] showed that hollow fibrin conduits supported muscle recovery and axonal growth in short nerve gaps. However, in longer nerve gaps, the hollow tubes failed in comparison to autografts. In larger nerve defects, the fibrin cable that facilitates SCs migration is not formed in the hollow NGTs. Therefore, NGTs are limited to nerve lesions < 4 cm and result in poor functional recovery at longer gaps.

Hydrogels as luminal fillers: The empty lumen of a nerve conduit lacks the necessary support structure for the ingrowth and migration of SCs and axons, thus making it an undesirable environment for axonal repair. Hence, in the longer nerve gaps, there is a need for a substrate inside the lumen of the NGTs that can provide necessary mechanical and biological cues for SCs migration and enhance nerve regeneration [36, 53]. Hydrogels are owing to their injectable behavior and their drug encapsulation and delivery capability have found their use as luminal fillers inside the conduits. Figure 5 shows how NGTs filled with hydrogels provide mechanical support in addition to serving as a carrier of bioactive molecules needed for proper functional recovery. This property is absent in the hollow tubes where bioactive cues are not present to direct proper axonal regeneration [54].

Various hydrogels alone or supplemented with small molecules, growth factors, neurotrophic factors, and cellular components have been used as luminal fillers for nerve conduits. For example, agarose hydrogels containing gradients of laminin-1 and nerve growth factor (NGF) molecules have been used in polysulfone (PSU) tubes [55]. Various researchers have also investigated the role of collagen as a luminal filler [53]. However, just the mere presence of these hydrogels sometimes is insufficient to achieve enhanced functional recovery. Therefore, luminal collagen fillers have been supplemented with laminin, NGF, fibroblast growth factor (FGF), etc. to promote better nerve regeneration. Similarly, fibrin gel has also been used to enhance SC migration, myelination, and rate of regeneration inside silicone tubes in a 1 cm rat sciatic nerve model [53].

Seckel et al. used hyaluronic acid gel in the conduits to produce better conduction velocity, higher axon counts, and myelination [56, 57]. They postulated that HA improves fibrin matrix formation and decreases scarring that might interfere with nerve regeneration. Mohammad et al. [58] showed that when HA was used with NGF, there was a 45% increase in the myelinated axon count. Most recently, keratin-based hydrogels that were used to fill commercial nerve tubes showed an improved axonal area and myelination compared to the empty tube. Electrophysiological analysis such as conduction delay and impulse amplitude were also better than the hollow tube and comparable to the autografts [59, 60]. Luminal fillers in nerve conduits supplemented with essential growth factors are promising ways to achieve nerve regeneration at par with autologous grafts. With an appropriate nerve conduit designed for long nerve gap, bioactive luminal fillers can aid in enhanced functional recovery.

Hydrogels have an added advantage in the field of peripheral nerve regeneration, as they can serve as a support system inside the conduit and also as a mode of delivery of various growth factors necessary for nerve regeneration. However, if the mechanical properties of the

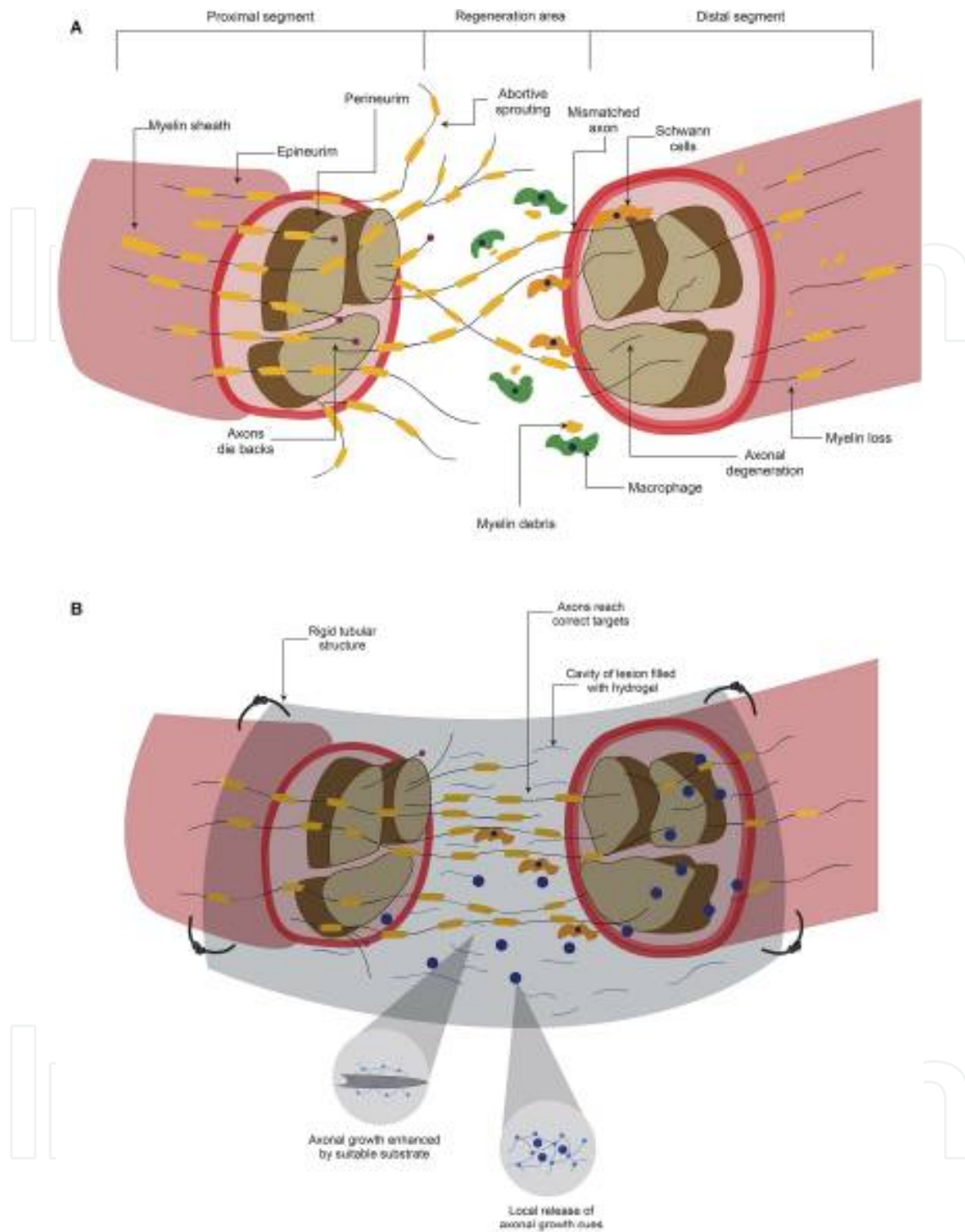


Figure 5. Hydrogels promote axonal regeneration after a peripheral nerve lesion. (A) After a lesion where peripheral nerves are severed, inhibitory elements for axonal regeneration arise either in proximal or in distal segments. Although there can be regeneration to unite both stumps, it is common that mismatches are formed. (B) When the lesion area is connected with a rigid tubular structure, and this is filled with a hydrogel, there is a mechanical support and a suitable substrate for axonal growth. In addition, the hydrogel can serve as a carrier of molecules that promote axonal regeneration and ultimately functional recovery. Reproduced from ref. [54] © Carballo-Molina and Velasco under the terms of Creative Commons Attribution License (CC BY).

hydrogels are not adjusted appropriately, they can hinder the nerve regeneration. Therefore, the limitation of using hydrogels as luminal fillers is primarily their cross-linking. Highly cross-linked viscous gels can be disadvantageous for nerve regeneration. At the same time, the rate of degradation of hydrogels plays an important role if they are used as conduit materials. Hence, it is essential to tune the mechanical and chemical properties of the hydrogels for their best use in peripheral nerve regeneration.

3.2. Hydrogels for tooth regeneration

Tooth regeneration similar to the construction of other tissues also requires an appropriate cell source, a biodegradable scaffold that can mimic the natural extracellular matrix (ECM) and bioactive molecules. Tooth organ is composed of enamel, dentin, cementum, and dental pulp. Cells such as ameloblasts form the enamel, odontoblasts form the dentin, cementoblasts form the cementum, and mesenchymal, fibroblastic, vascular, and neural cells form the dental pulp [61]. Scaffold materials play a critical role in determining how cells proliferate and differentiate. Those that mimic the characteristics of natural ECM can best promote appropriate cell and tissue maturation. The tooth scaffolds should be such that they provide chemical and mechanical integrity, are biocompatible, are able to restore the normal functioning of the tooth, and are able to integrate with the surrounding tissues [25]. For dentin-pulp tissue engineering, in particular, hydrogels come across as a favorable choice because they are injectable and have a 3D morphology that helps in the encapsulation of cells and growth factors. Hydrogel scaffolds made from natural biopolymers such as collagen, chitosan, hyaluronic acid, gelatin, fibrin, and alginate have been used quite extensively since they are readily cross-linkable and can be easily combined with various bioactive molecules [62]. Kim et al. [63] loaded collagen gels with a series of growth factors and injected them into pulp chambers and root canals of endodontically treated human teeth. They found that on *in vivo* implantation of endodontically treated human teeth in mouse dorsum for the tested 3 or 6 weeks, there was a recellularized and revascularized connective tissue that integrated to the native dentinal wall in root canals.

Collagen gels have also been used to deliver dental pulp stem cells (DPSCs) and dentin matrix protein-1 (DMP-1) *in vivo* where it led to the ectopic formation of dental pulp-like tissue [64]. Although, collagen is a major ECM component used to fabricate hydrogel scaffolds in tissue engineering, its moderate mechanical strength is a limitation [61]. For this reason, Bhatnagar et al. [25] used cross-linked gelatin scaffolds for dentin regeneration without any external chemical stimulus. These gelatin hydrogel scaffolds that were cross-linked with microbial transglutaminase (mTG) provided variable mechanical properties and were capable of differentiating DPSCs *in vitro* toward odontogenesis irrespective of their mechanical stimulus and external chemical inducer (Figure 6). Alginate hydrogels have also been used for dentin-pulp regeneration. When loaded with exogenous transforming growth factor TGF β 1, these hydrogels were shown to promote dentin matrix secretion and odontoblast-like cell differentiation [65]. Among synthetic polymers, PLGA hydrogels with recombinant human growth differentiation factor-5 (rhGDF-5) have been used in periodontal defects in a dog model. Periodontal pockets (3 \times 6mm, width \times depth) were surgically created over the buccal roots of

the second and fourth mandibular premolars in mongrel dogs and progressive alveolar bone maturation was seen at 6 weeks on rhGDF-5/PLGA delivery [66].

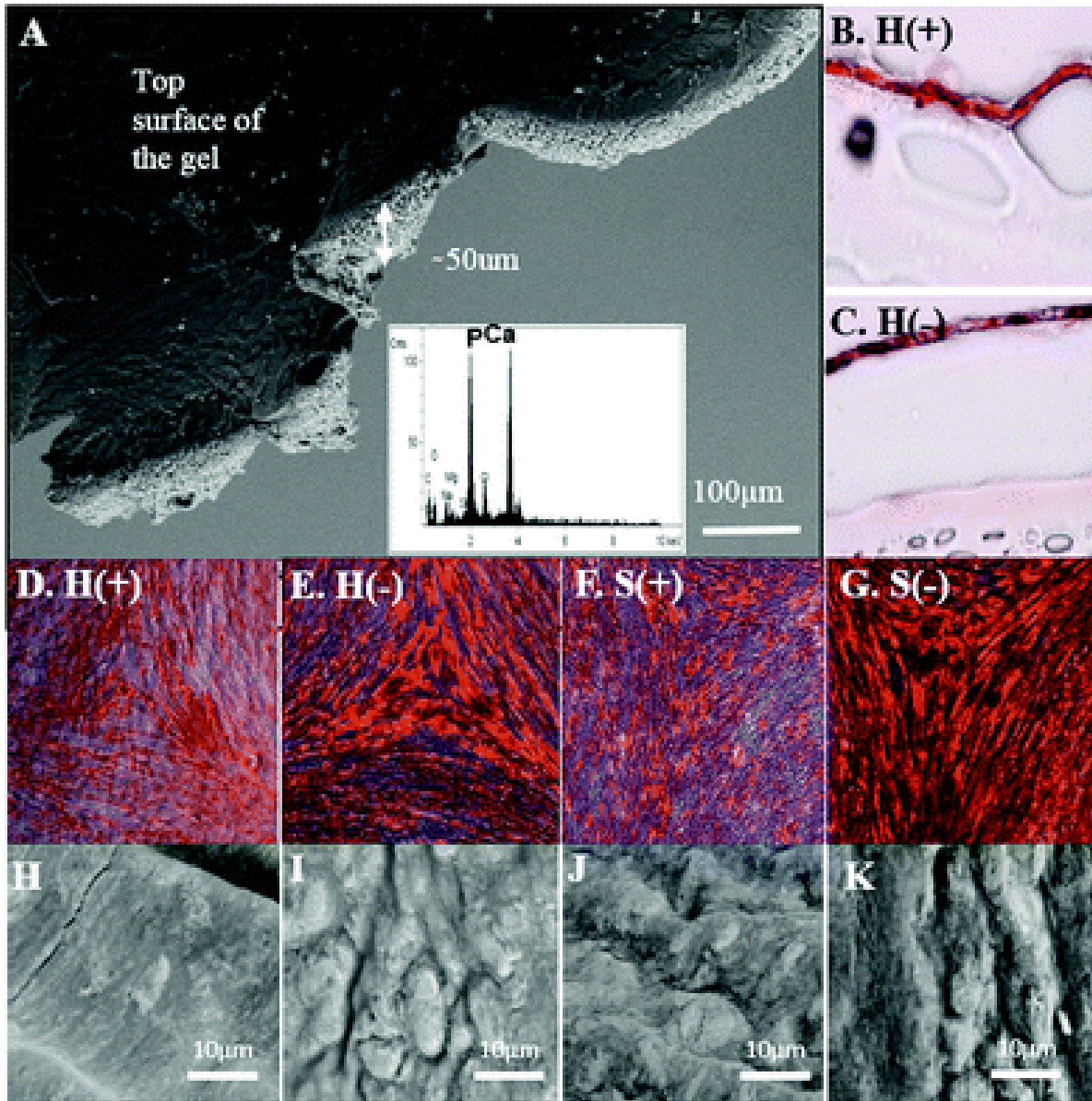


Figure 6. (A) Cross-section of a non-induced hard gel (H (-)) after 35 days of DPSCs culture showing a self-supporting sheet of biomineralized deposits present inside the gel. EDX spectra (inset in (A)) confirm the hydroxyapatite mineral. A cross-sectional view of the alizarin red-stained calcified biomineralized deposits in the (B) hard (+) and (C) hard (-) gel. Top view of the alizarin red-stained calcified deposits and their corresponding SEM images after 35 days of DPSCs cultured on: (D, H) hard (+); (E, I) hard (-); (F, J) soft (+); (G, K) soft (-) gels. The calcified deposits laid by the cells are stained dark red and have a defined pattern. Reproduced from ref. [25] © by permission of The Royal Society of Chemistry.

Hydrogels have shown their potential in regenerating dentin-pulp tissue. Researchers have demonstrated the successful use of hydrogel scaffolds for dentin-pulp matrix regeneration. However, hydrogels have a limitation when it comes to regenerating the whole tooth organ.

Not much research has been done in the field of using hydrogel scaffolds for regenerating the whole tooth structure.

3.3. Hydrogels for 3D printing

3D printing is emerging as a potential tool in regenerative medicine for building complex 3D structures across length scales ranging from micrometers to millimeters. 3D printing represents a way to pattern and assemble the cells with materials in a controlled and functional 3D architecture. The only limitation that arises is due to the materials being printed and necessitates a need for new inks to expand the utility of 3D printed structures [67]. 3D printing techniques generally comprises of: (a) extrusion-based printing that requires a material to be extruded through an orifice, (b) ink-jet based printing that requires a material to be ejected as droplets onto a substrate, and (c) laser based printing where a material is cured using a laser [67].

Hydrogels for 3D printing should be printable, biocompatible, have desired mechanical properties, shape, and structure (Table 2) [68]. Collagen has been extensively used for 3D printing where in one case, sodium hydrogen carbonate (NaHCO_3) vapor was applied to gel the printed collagen layer and in another instance, NaHCO_3 was mixed with collagen and cells and then printed using laser-assisted bioprinting [68]. Several researchers have utilized the temperature-responsive hydrogels, particularly pluronic F127 that gels in the temperature range of 10 to 40°C. Pluronics have been combined with collagen and cross-linked gelatin methacrylate (GelMa) to form bioinks. Kolesky et al. printed pluronic F127 as a sacrificial vascular network embedded in GelMa matrix that mimic natural fine capillaries [69].

Ideal bioprinting hydrogel properties	
Printability	Viscosity
	Shear-thinning property
	Response and transition time
	Sol-gel transition stimulus
Biocompatibility	Degradability
	Cell-binding motifs
	Non-toxic
	Non-immunogenic
Mechanical Properties	Stiffness
	Elasticity
	Strength
Shape and structure	Pore size
	Micro/Nanostructure

Table 1. Ideal bioprinting hydrogel properties. Reproduced from ref. [68] © Wang et al., under the terms of Creative Commons Attribution-Non-commercial 4.0 International License.

Photocross-linking property of the hydrogels has been utilized to bioprint tough and rigid hydrogel constructs with cells. For example, partially photocross-linking gelatin methacry-

late (GelMA) was combined with hyaluronic acid methacrylate (HAMA) to form a gel-like fluid which was then printed with a defined pattern. This printed layer was further irradiated to obtain a tubular tissue construct [70]. Hong et al. [71] combined sodium alginate and poly (ethylene glycol) (PEG) to constitute an interpenetrating network. Laponite clay was used to form a nanogel. Poly(ethylene glycol) diacrylate (PEGDA) and alginate mixture were combined with laponite clay to form a pre-gel solution. To cross-link PEGDA and alginate, a photoinitiator and calcium sulfate solution were added to the pre-gel solution. The PEGDA–alginate–nanoclay pre-gel solution was 3D printed via extrusion-based printing (Figure 7). The resulting hydrogels were tough and had the potential to encapsulate cells for tissue regeneration.

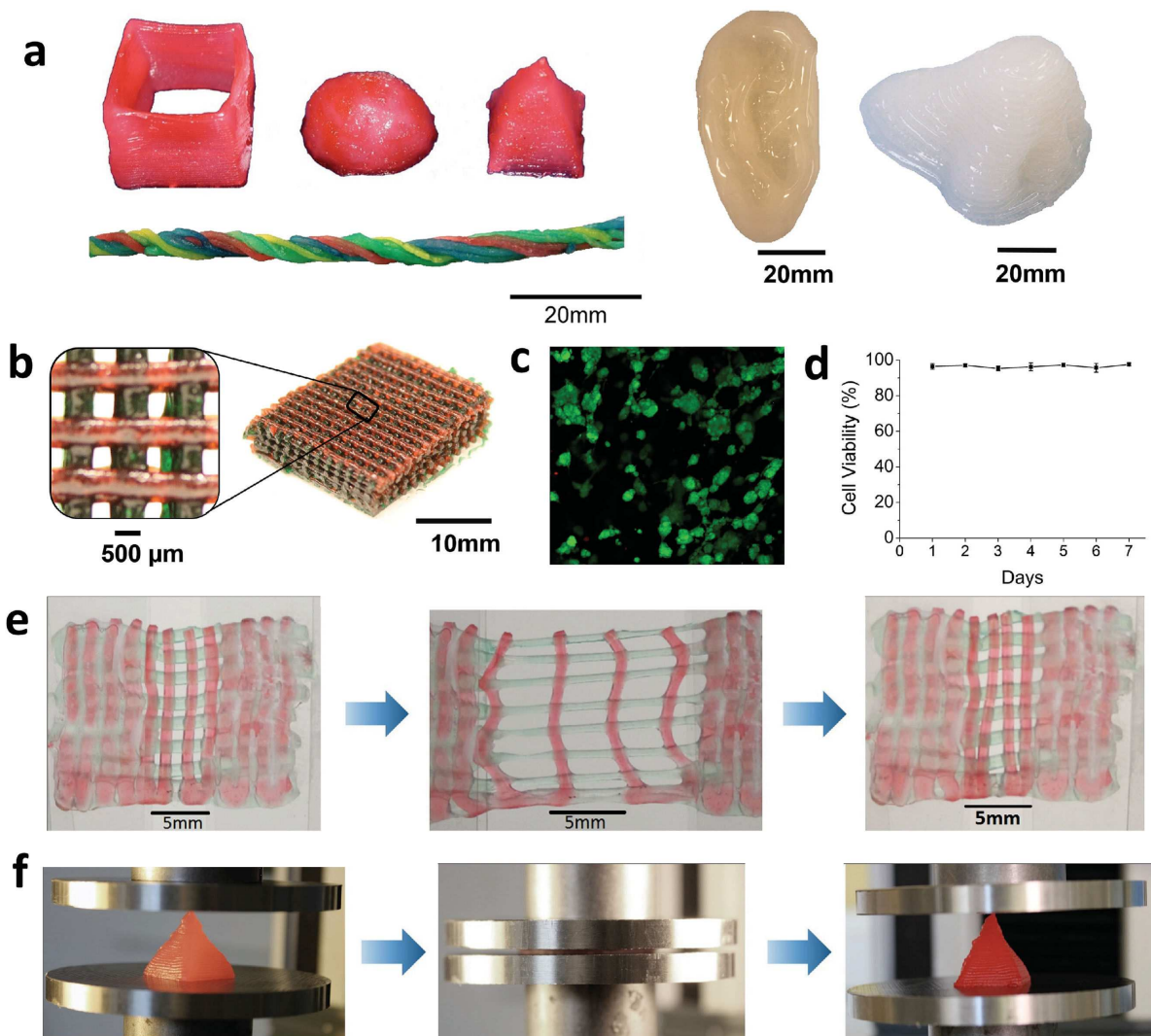


Figure 7. 3D printing of tough and biocompatible PEG–alginate–nanoclay hydrogels. (a) Various 3D constructs printed with the hydrogel (from left to right: hollow cube, hemisphere, pyramid, twisted bundle, the shape of an ear, and a nose. Non-toxic red food dye was added postprint on some samples for visibility). (b) A mesh printed with the tough and biocompatible hydrogel. The mesh was used to host HEK cells. (c) Live–dead assay of HEK cells in a collagen hydrogel infused into the 3D printed mesh of the PEG–alginate–nanoclay hydrogel. (d) The viability of the HEK cells through 7 d. (e) A printed bilayer mesh (top layer red, bottom layer green) is uniaxially stretched to three times its

initial length. Relaxation of the sample after stretching shows almost complete recovery of its original shape. (f) A printed pyramid undergoes a compressive strain of 95% while returning to its original form after relaxation. Reproduced with permission from ref. [71] © John Wiley and Sons.

Recent developments in 3D printing of hydrogels offer a potential to produce constructs with the higher structural organization, fine-tuned mechanical and chemical properties to control cell behavior and an environment that mimics *in vivo* tissue. 3D printing of hydrogels is promising and requires further development such that the hydrogels are easy and inexpensive to print, are favorable toward promoting cell viability, differentiation, migration, and cell–cell interactions, and are functionally versatile. However, hydrogels are soft, and their use for 3D printing largely depends on their viscosity, their structural integrity, and their ability to be cross-linked in a way such that the cells can be encapsulated.

4. Conclusion

Hydrogels have found extensive applicability in various fields of tissue engineering and regenerative medicine due to their underlying similarity to the native ECM. The role of hydrogels in regenerative medicine has progressed remarkably with their widespread use in peripheral nerve regeneration, tooth regeneration, and more recently in 3D printing. Long nerve gap repair, dentin-pulp complex reconstruction, and 3D printing of organs are few of the areas in regenerative medicine that are at the forefront. Understanding and development of functionally bioactive smart hydrogels could help tremendously in these regenerative therapies.

Acknowledgements

This work was supported by NSF-Inspire Program grant # DMR- 1344267.

Author details

Divya Bhatnagar^{1*}, Marcia Simon² and Miriam H. Rafailovich³

*Address all correspondence to: divya.bhatnagar22@gmail.com

1 New Jersey Center for Biomaterials, Rutgers University, Piscataway, NJ, USA

2 Department of Oral Biology and Pathology, Stony Brook School of Dental Medicine, Stony Brook, NY, USA

3 Department of Materials Science and Engineering, Stony Brook University, Stony Brook, NY, USA

References

- [1] Tibbitt, M.W. and K.S. Anseth, *Hydrogels as extracellular matrix mimics for 3D cell culture*. *Biotechnology and Bioengineering*, 2009. 103(4): pp. 655–663.
- [2] Hoffman, A.S., *Hydrogels for biomedical applications*. *Advanced Drug Delivery Reviews*, 2012. 64: pp. 18–23.
- [3] Cabral, J. and S.C. Moratti, *Hydrogels for biomedical applications*. *Future Medicinal Chemistry*, 2011. 3(15): pp. 1877–1888.
- [4] Nayak, S. and L.A. Lyon, *Soft nanotechnology with soft nanoparticles*. *Angewandte Chemie International Edition*, 2005. 44(47): pp. 7686–7708.
- [5] Ghosh, K., et al., *Cell adaptation to a physiologically relevant ECM mimic with different viscoelastic properties*. *Biomaterials*, 2007. 28: pp. 671–679.
- [6] Bhatnagar, D., et al., *Rheological Characterization of Novel HA-Pluronic Thermoreversible Hydrogels*. *Journal of Chemical and Biological Interfaces*, 2013. 1(2): pp. 93–99.
- [7] Bhatnagar, D., et al., *Hyaluronic Acid and Gelatin Clay Composite Hydrogels: Substrates for Cell Adhesion and Controlled Drug Delivery*. *Journal of Chemical and Biological Interfaces*, 2014. 2(1): pp. 34–44.
- [8] Hennink, W. and C.F. Van Nostrum, *Novel crosslinking methods to design hydrogels*. *Advanced Drug Delivery Reviews*, 2012. 64: pp. 223–236.
- [9] Campoccia, D., et al., *Semisynthetic resorbable materials from hyaluronan esterification*. *Biomaterials*, 1998. 19(23): pp. 2101–2127.
- [10] Prestwich, G.D., et al., *Controlled chemical modification of hyaluronic acid: synthesis, applications, and biodegradation of hydrazide derivatives*. *Journal of Controlled Release*, 1998. 53(1): pp. 93–103.
- [11] Nayak, S. and L.A. Lyon, *Soft Nanotechnology with Soft Nanoparticles*. *Angewandte Chemie International Edition*, 2005. 44: pp. 7686–7708.
- [12] Lee, K., *DJ Mooney Hydrogels for tissue engineering*. *Chemistry Review*, 1869. 101: pp. 2001.
- [13] Ghosh, K., et al., *Rheological Characterization of in Situ Cross-Linkable Hyaluronan Hydrogels*. *BioMacromolecules*, 2005. 6(5): pp. 2857–2865.
- [14] Rafailovich, M., D. Bhatnagar, and M.K. Cowman, *Nanocomposite hyaluronic acid-clay based hydrogels*. 2013, Google Patents.
- [15] Watanabe, T., et al., *NMR studies on water and polymer diffusion in dextran gels. Influence of potassium ions on microstructure formation and gelation mechanism*. *Magnetic Resonance in Medicine*, 1996. 35(5): pp. 697–705.

- [16] Huang, S. and X. Fu, *Naturally Derived Materials-Based Cell and Drug Delivery Systems in Skin Regeneration*. *Journal of Controlled Release*, 2010. 142(2): pp. 149–159.
- [17] Jiang, J., et al., *Rheology of thermoreversible hydrogels from multiblock associating copolymers*. *Macromolecules*, 2008. 41(10): pp. 3646–3652.
- [18] Eagland, D., N. Crowther, and C. Butler, *Complexation between polyoxyethylene and polymethacrylic acid—the importance of the molar mass of polyoxyethylene*. *European Polymer Journal*, 1994. 30(7): pp. 767–773.
- [19] Mathur, A.M., et al., *Equilibrium swelling of poly (methacrylic acid-g-ethylene glycol) hydrogels: Effect of swelling medium and synthesis conditions*. *Journal of Controlled Release*, 1998. 54(2): pp. 177–184.
- [20] Wichterle, O. and D. Lim, *Hydrophilic gels for biological use*. 1960.
- [21] Gulrez, S.K., G.O. Phillips, and S. Al-Assaf, *Hydrogels: methods of preparation, characterisation and applications*. 2011: INTECH Open Access Publisher.
- [22] Ratner, B.D., et al., *Biomaterials science: an introduction to materials in medicine*. 2004: Academic press.
- [23] Yung, C., et al., *Transglutaminase crosslinked gelatin as a tissue engineering scaffold*. *Journal of Biomedical Materials Research Part A*, 2007. 83(4): pp. 1039–1046.
- [24] Ito, A., et al., *Transglutaminase-mediated gelatin matrices incorporating cell adhesion factors as a biomaterial for tissue engineering*. *Journal of Bioscience and Bioengineering*, 2003. 95(2): pp. 196–199.
- [25] Bhatnagar, D., et al., *Biomineralization on enzymatically cross-linked gelatin hydrogels in the absence of dexamethasone*. *Journal of Materials Chemistry B*, 2015.
- [26] Bettini, R., P. Colombo, and N.A. Peppas, *Solubility effects on drug transport through pH-sensitive, swelling-controlled release systems: Transport of theophylline and metoclopramide monohydrochloride*. *Journal of Controlled Release*, 1995. 37(1): pp. 105–111.
- [27] Cicek, H. and A. Tuncel, *Immobilization of α -chymotrypsin in thermally reversible isopropylacrylamide-hydroxyethylmethacrylate copolymer gel*. *Journal of Polymer Science Part A: Polymer Chemistry*, 1998. 36(4): pp. 543–552.
- [28] Xu, D., et al., *Rheology of Poly (N-isopropylacrylamide)–Clay Nanocomposite Hydrogels*. *Macromolecules*, 2015. 48(3): pp. 840–846.
- [29] Lombardi, J., et al., *Rheology of Glycated Poly (N-isopropylacrylamide)–Clay Nanogels*. *Journal of Chemical and Biological Interfaces*, 2014. 2(1): pp. 45–49.
- [30] Haraguchi, K., et al., *Mechanism of forming organic/inorganic network structures during in-situ free-radical polymerization in PNIPA-clay nanocomposite hydrogels*. *Macromolecules*, 2005. 38(8): pp. 3482–3490.

- [31] Haraguchi, K. and T. Takehisa, *Nanocomposite hydrogels: a unique organic-inorganic network structure with extraordinary mechanical, optical, and swelling/de-swelling properties*. *Advanced Materials*, 2002. 14(16): p. 1120.
- [32] Miyazaki, S., et al., *Gelation mechanism of poly (N-isopropylacrylamide)-clay nanocomposite gels*. *Macromolecules*, 2007. 40(12): pp. 4287–4295.
- [33] Haraguchi, K., et al., *Compositional effects on mechanical properties of nanocomposite hydrogels composed of poly (N, N-dimethylacrylamide) and clay*. *Macromolecules*, 2003. 36(15): pp. 5732–5741.
- [34] Langer, R. and J. Vacanti, *Tissue engineering*. *Science*, 1993. 14(260(5110)): pp. 920–926.
- [35] Slaughter, B.V., et al., *Hydrogels in Regenerative Medicine*. *Advanced Materials*, 2009. 21: pp. 3307–3329.
- [36] Pabari, A., et al., *Recent advances in artificial nerve conduit design: strategies for the delivery of luminal fillers*. *Journal of Controlled Release*, 2011. 156(1): pp. 2–10.
- [37] Kehoe, S., X. Zhang, and D. Boyd, *FDA approved guidance conduits and wraps for peripheral nerve injury: a review of materials and efficacy*. *Injury*, 2012. 43(5): pp. 553–572.
- [38] Khaing, Z.Z. and C.E. Schmidt, *Advances in natural biomaterials for nerve tissue repair*. *Neuroscience Letters*, 2012. 519(2): pp. 103–114.
- [39] Meek, M.F. and J.H. Coert, *US Food and Drug Administration/Conformit Europe-approved absorbable nerve conduits for clinical repair of peripheral and cranial nerves*. *Annals of Plastic Surgery*, 2008. 60(1): pp. 110–116.
- [40] Archibald, S., et al., *Monkey median nerve repaired by nerve graft or collagen nerve guide tube*. *The Journal of Neuroscience*, 1995. 15(5): pp. 4109–4123.
- [41] Archibald, S.J., et al., *A collagen-based nerve guide conduit for peripheral nerve repair: An electrophysiological study of nerve regeneration in rodents and nonhuman primates*. *Journal of Comparative Neurology*, 1991. 306(4): pp. 685–696.
- [42] Farole, A. and B.T. Jamal, *A bioabsorbable collagen nerve cuff (NeuraGen) for repair of lingual and inferior alveolar nerve injuries: a case series*. *Journal of Oral and Maxillofacial Surgery*, 2008. 66(10): pp. 2058–2062.
- [43] Li, S.-T., et al., *Peripheral nerve repair with collagen conduits*. *Clinical materials*, 1992. 9(3): pp. 195–200.
- [44] Gu, X., et al., *Construction of tissue engineered nerve grafts and their application in peripheral nerve regeneration*. *Progress in Neurobiology*, 2011. 93(2): pp. 204–230.
- [45] Phillips, J.B., et al., *Neural tissue engineering: a self-organizing collagen guidance conduit*. *Tissue Engineering*, 2005. 11(9-10): pp. 1611–1617.

- [46] Sakai, Y., et al., *New artificial nerve conduits made with photocrosslinked hyaluronic acid for peripheral nerve regeneration*. *Bio-Medical Materials and Engineering*, 2007. 17(3): pp. 191–197.
- [47] Jansen, K., et al., *A hyaluronan-based nerve guide: in vitro cytotoxicity, subcutaneous tissue reactions, and degradation in the rat*. *Biomaterials*, 2004. 25(3): pp. 483–489.
- [48] Ornelas, L., et al., *Fibrin glue: an alternative technique for nerve coaptation-Part II. Nerve regeneration and histomorphometric assessment*. *Journal of Reconstructive Microsurgery*, 2006. 22(2): pp. 123–128.
- [49] di Summa, P.G., et al., *Long-term in vivo regeneration of peripheral nerves through bioengineered nerve grafts*. *Neuroscience*, 2011. 181: pp. 278–291.
- [50] Kalbermatten, D.F., et al., *New fibrin conduit for peripheral nerve repair*. *Journal of Reconstructive Microsurgery*, 2009. 25(1): p. 27–33.
- [51] Pettersson, J., et al., *Biodegradable fibrin conduit promotes long-term regeneration after peripheral nerve injury in adult rats*. *Journal of Plastic, Reconstructive & Aesthetic Surgery*, 2010. 63(11): pp. 1893–1899.
- [52] Pettersson, J., et al., *Muscle recovery after repair of short and long peripheral nerve gaps using fibrin conduits*. *Neuroscience Letters*, 2011. 500(1): pp. 41–46.
- [53] Chen, M.B., F. Zhang, and W.C. Lineaweaver, *Luminal fillers in nerve conduits for peripheral nerve repair*. *Annals of Plastic Surgery*, 2006. 57(4): pp. 462–471.
- [54] Carballo-Molina, O.A. and I. Velasco, *Hydrogels as scaffolds and delivery systems to enhance axonal regeneration after injuries*. *Frontiers in Cellular Neuroscience*, 2015. 9.
- [55] Dodla, M.C. and R.V. Bellamkonda, *Differences between the effect of anisotropic and isotropic laminin and nerve growth factor presenting scaffolds on nerve regeneration across long peripheral nerve gaps*. *Biomaterials*, 2008. 29(1): pp. 33–46.
- [56] Seckel, B., et al., *Hyaluronic acid through a new injectable nerve guide delivery system enhances peripheral nerve regeneration in the rat*. *Journal of Neuroscience Research*, 1995. 40(3): pp. 318–324.
- [57] Wang, K.K., et al., *Hyaluronic acid enhances peripheral nerve regeneration in vivo*. *Microsurgery*, 1998. 18(4): pp. 270–275.
- [58] Mohammad, J.A., et al., *Increased axonal regeneration through a biodegradable amnionic tube nerve conduit: effect of local delivery and incorporation of nerve growth factor/hyaluronic acid media*. *Annals of Plastic Surgery*, 2000. 44(1): pp. 59–64.
- [59] Apel, P.J., et al., *Peripheral nerve regeneration using a keratin-based scaffold: long-term functional and histological outcomes in a mouse model*. *The Journal of Hand Surgery*, 2008. 33(9): pp. 1541–1547.

- [60] Hill, P.S., et al., *Repair of peripheral nerve defects in rabbits using keratin hydrogel scaffolds*. *Tissue Engineering Part A*, 2011. 17(11–12): pp. 1499–1505.
- [61] Yuan, Z., et al., *Biomaterial selection for tooth regeneration*. *Tissue Engineering Part B: Reviews*, 2011. 17(5): pp. 373–388.
- [62] Laurenti, M. and M.-N. Abdallah, *Natural and synthetic hydrogels for periodontal tissue regeneration*. *International Dental Journal of Students Research*, 2015. 3(2): pp. 49–51.
- [63] Kim, J.Y., et al., *Regeneration of dental-pulp-like tissue by chemotaxis-induced cell homing*. *Tissue Engineering Part A*, 2010. 16(10): pp. 3023–3031.
- [64] Huang, G.T.-J., et al., *Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model*. *Tissue Engineering Part A*, 2009. 16(2): pp. 605–615.
- [65] Dobie, K., et al., *Effects of alginate hydrogels and TGF- β 1 on human dental pulp repair in vitro*. *Connective Tissue Research*, 2002. 43(2–3): pp. 387–390.
- [66] Kwon, D.H., et al., *Evaluation of an injectable rhGDF-5/PLGA construct for minimally invasive periodontal regenerative procedures: a histological study in the dog*. *Journal of Clinical Periodontology*, 2010. 37(4): pp. 390–397.
- [67] Highley, C.B., C.B. Rodell, and J.A. Burdick, *Direct 3D Printing of Shear-Thinning Hydrogels into Self-Healing Hydrogels*. *Advanced Materials*, 2015. 27(34): pp. 5075–5079.
- [68] Wang, S., J.M. Lee, and W.Y. Yeong, *Smart hydrogels for 3D bioprinting*. *International Journal of Bioprinting*, 2015. 1(1).
- [69] Kolesky, D.B., et al., *3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs*. *Advanced Materials*, 2014. 26(19): pp. 3124–3130.
- [70] Skardal, A., et al., *Photocrosslinkable hyaluronan-gelatin hydrogels for two-step bioprinting*. *Tissue Engineering Part A*, 2010. 16(8): pp. 2675–2685.
- [71] Hong, S., et al., *3D Printing of Highly Stretchable and Tough Hydrogels into Complex, Cellularized Structures*. *Advanced Materials*, 2015. 27(27): pp. 4035–4040.

