

# Nanostructured Hydrogels for Tissue Engineering and Regenerative Medicine

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## Introduction

In recent years there has been a demand for engineered functional tissues which would mimic body's intricate tissue architecture and also maintain necessary microenvironment for cellular growth. A challenge for designing such functional tissue is the development of biomaterials with controlled mechanical, physical, chemical and biological properties. Among various biomaterials currently available hydrogels are the most suited for this application as they closely simulate native tissue present inside the body. Hydrogels consist of three-dimensional (3D) crosslinked polymeric network which can absorb large quantities of water, giving some unique properties to these materials. Hydrogels are synthesized from natural or synthetic polymers and possess various advantages over conventional organic and inorganic materials such as biodegradability, biocompatibility, processability, and functionalization. But the conventional polymeric hydrogels lack necessary mechanical strength, structural stability, electrical and magnetic properties which limit their application.

To overcome conventional limitations, a range of nanoparticles including metallic, inorganic, organic or polymeric nanomaterials, are incorporated within polymeric hydrogels to improve and impart tailored properties to crosslinked network. These nanomaterials strengthen the polymer matrix leading to nanocomposite hydrogels offering enhanced mechanical, electrical, optical, magnetic and biomedical properties. The various properties of the nanocomposite hydrogels can be tailored by selecting different combinations of polymer and nanomaterial. The nanomaterials can interact covalently or physically with the polymeric networks and provide tunable functionalities to the nanocomposite hydrogels. For example, addition of multiwall carbon nanotube (mCNTs) or gold nanowires within polymeric hydrogel render formation of electrically conductive network that can be used to engineer cardiac patch or muscle tissues.

There has been a growing interest in tailoring these nanostructured hydrogels for tissue engineering applications. The primary goal of tissue engineering is to repair or replace biological tissue or organs with the help of tissue scaffold. Cellular proliferation, adhesion, migration and signaling are some of the important functionalities that are required in these engineered tissues so that they can be used to treat organ failures resulting from diseases and injuries. Recent advances in biomaterials development have focused on engineering multifunctional biomaterials to control and direct cell functions. The essential objective accomplished by a tissue scaffold is it incorporates biomaterials which can replicate the complex biological and mechanical tissue microenvironment enabling regenerative tissue growth.

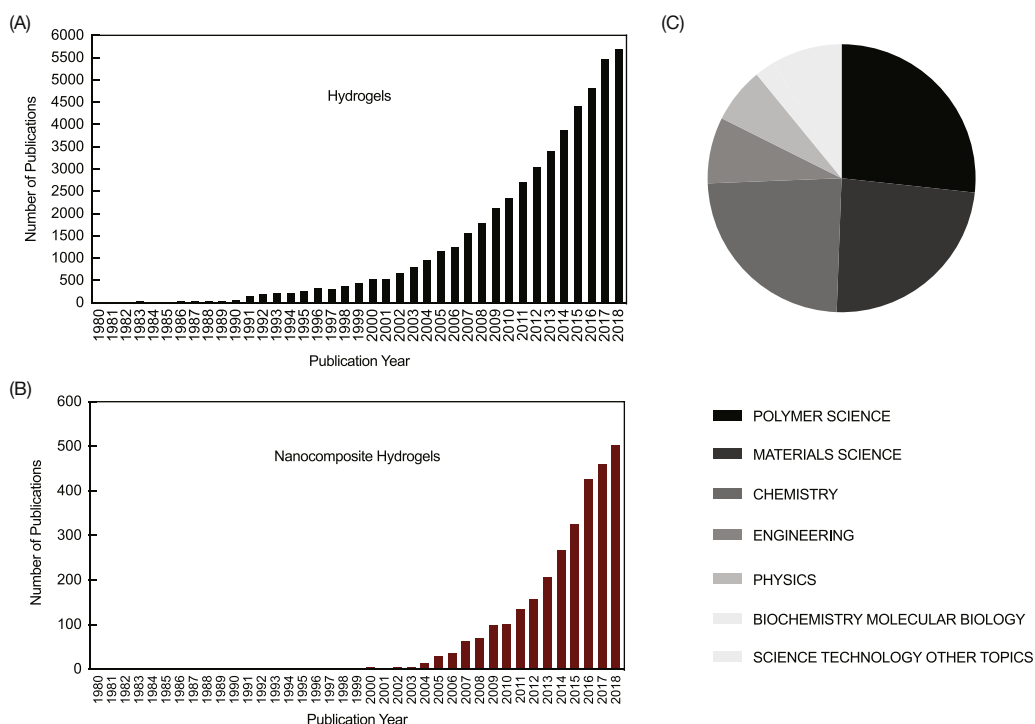
In the last decade nanomaterials have become attractive for biomedical applications due to their small size, enhanced mechanical properties, high surface area-to-volume ratio, tunable optical and magnetic properties. Compared to microscale particles, nanoparticles below 50 nm of size can easily enter cells and nanoparticles below 20 nm of size can move out of blood vessels which enables easy movement of the particles to different organs and tissues. This movement aids in interaction of the particles with both intracellular and extracellular components. Due to the enhanced access the nanomaterials have to various components of the body, they have potential to deliver therapeutics, monitor cells, act as imaging agents and be involved in photodynamic therapy.

Size, shape and surface properties are crucial in controlling biomedical application of various nanomaterials which renders unique properties to the nanostructured hydrogels. Size is critical in controlling the surface-to-volume ratio which governs its biodistribution and uptake throughout the body. Size is crucial when application of nanoparticle is specific such as for targeted drug delivery. Shape of nanoparticles is again crucial in deciding their cellular uptake and interactions with specific proteins. Gold (Au) nanoparticle uptake is enhanced when they are spherical in shape and above 50 nm in size. The change in shape of the Au NPs from spherical to rod like enables the surface plasma resonance (SPR) to be in the Near Infrared (NIR) window enabling their usage for in vivo imaging and NIR responsive drug release, making them an attractive choice for clinical applications.

There has been a continued research interest in nanocomposite hydrogels which is evident through the number of publications and diverse research fields working in this area (Fig. 1). In the following section, we will discuss different types of nanoengineered biomaterials for tissue engineering and regenerative medicine. Specifically, we will explain the unique characteristics imparted by various nanomaterials such as metallic, carbon-based, inorganic and organic nanoparticles within polymeric hydrogels. Some of the emerging applications of these nanoengineered hydrogels with respect to therapeutic delivery, imaging, tissue engineering, organoids, and bioprinting (Fig. 2) will be discussed.

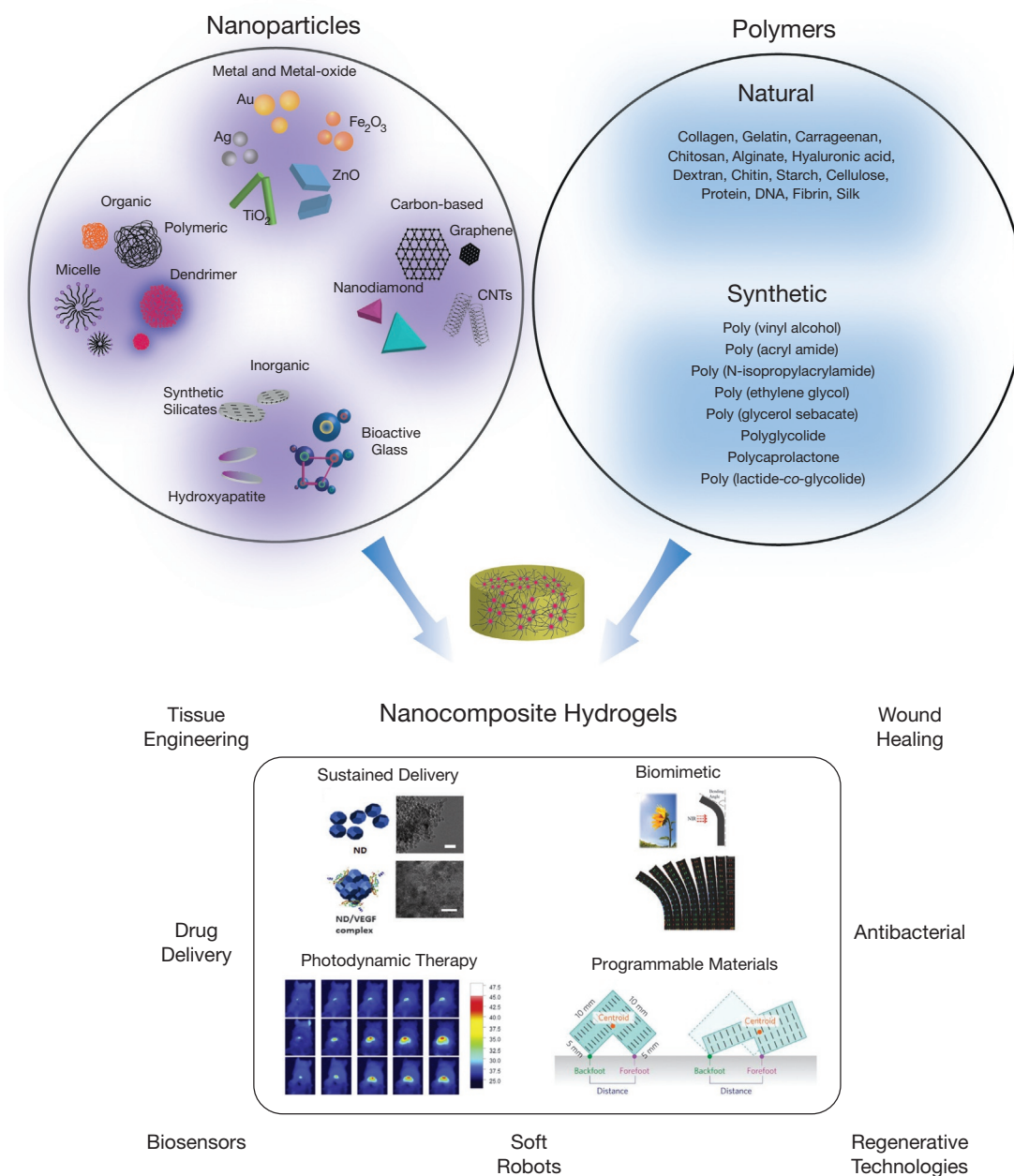
### Metal and Metal-Oxide Nanoparticle Based Nanocomposite Hydrogels

Metallic nanoparticles include silver (Ag), platinum (Pt), gold (Au) and other noble elements, whereas metal-oxide nanoparticles comprise of iron oxide ( $\text{Fe}_2\text{O}_3$ ,  $\text{Fe}_3\text{O}_4$ ), alumina ( $\text{Al}_2\text{O}_3$ ), titania ( $\text{TiO}_2$ ), zinc oxide (ZnO), and copper oxide (CuO). These nanoparticles are combined with polymeric network through covalent or non-covalent integration to synthesize nanocomposite hydrogels, rendering unique properties such as electrical conductivity, magnetic properties, enhanced mechanical strength and stimuli responsiveness to the nanocomposite hydrogels. These unique properties enable the nanostructures to be used in biosensors, imaging agents, responsive scaffolds, and drug delivery systems.



**Fig. 1** Number of publications in the field of (A) hydrogels and (B) nanocomposite hydrogels according to ISI Web of Science (data obtained on Jan 2019). There has been a steady increase in the number of publications indicating growing interest in the field of nanocomposite hydrogels. (C) Research areas working in field of nanocomposite hydrogels.

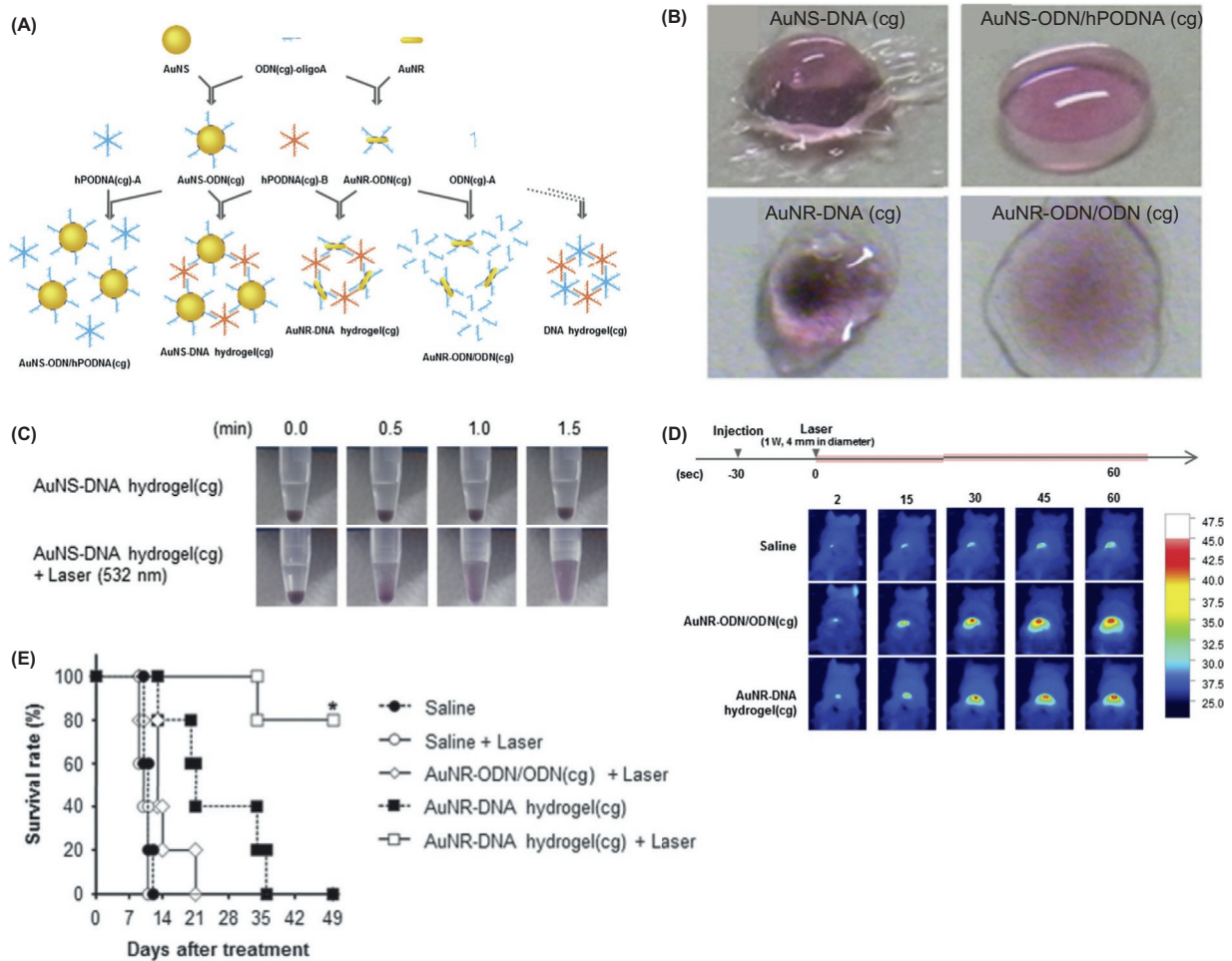
## Nanocomposite Hydrogels for Biomedical Applications



**Fig. 2** Designing nanocomposite hydrogels with tunable functionalities for diverse applications.

### Gold Nanoparticles

Au nanoparticles (NPs) have been used to synthesize hydrogels having various functionalities. Au NPs exhibit localized surface plasmon resonance and convert optical energy into thermal energy. This makes the Au-nanocomposites suitable candidates for photodynamic therapy, photo thermal therapy, imaging and stimuli responsive drug release. Au-nano spheres and Au-nanorods have been utilized to conceive unique composite DNA hydrogel systems for photothermal cancer immunotherapy (Fig. 3A). The Au nanoparticles are modified with oligodeoxynucleotide and hexapod-like structured DNA (hexapodna) due to their photosensitive nature and affinity to DNA (Fig. 3B). The hydrogel was irradiated with NIR laser (Fig. 3C) resulting in release of hexapodna (Fig. 3D). This lead to stimulation of immune cells and release of inflammatory cytokines (TNF- $\alpha$  and (IL)-6) arresting tumor growth with extended survival of the tumor bearing mice during treatment (Fig. 3E). The particular technique utilizes dual treatment approach to combat cancer which includes photothermal ablation and stimulation of immune system.



**Fig. 3** Gold nanoparticle-immunostimulatory DNA hydrogel. (A) Schematic illustrating synthesis of various AuNS-DNA and AuNR-DNA hydrogels. (B) Optical images of AuNS-DNA (cg), AuNS-ODN/hPODNA (cg), AuNR-DNA (cg), AuNR-ODN/ODN (cg) hydrogels. (C) Physical appearance of AuNS-hydrogel (cg) with or without laser irradiation. (D) Thermographic images of mice after laser irradiation. (E) Survival rate of tumor bearing mice during treatment. Adapted and reproduced by permission from Elsevier (Yata, T., Takahashi, Y., Tan, M., Nakatsuji, H., Ohtsuki, S., et al., (2017). DNA nanotechnology-based composite-type gold nanoparticle-immunostimulatory DNA hydrogel for tumor photothermal immunotherapy. *Biomaterials* **146**, 136–145) © 2017.

Au NPs have also been used in hydrogels where they act as crosslinking agents and enhance the physical, chemical, mechanical and biological properties of the nanocomposites. Thiol-functionalized Au NPs have been used to crosslink nanocomposite hydrogels consisting of gelatin and hyaluronic acid (HA) wherein the crosslink density is enhanced giving rise to improved stiffness of the hydrogel. Gold nanowires have been used in alginate scaffolds improving overall mechanical properties and electrical conductivity of the alginate hydrogel scaffolds making them a better platform for seeding cardiac cells as the cells exhibited better alignment rather than pure alginate scaffolds where the cells formed aggregates. For example, pure alginate scaffolds exhibited a porous structure wherein the porosities acted as resistance to electrical conduction but in alginate scaffolds reinforced with gold nanowires, the nanowires bridged the porosities making the scaffold more conducting than ones without nanowire reinforcement. Thus, when Au NPs reinforced alginate patches seeded with cardiac cells were electrically stimulated, the cells performed a synchronized contraction and relaxation.

### Silver Nanoparticles

Ag NPs are widely used for their antimicrobial properties. Ag nanoparticle loaded quaternised polyvinyl alcohol (PVA) hydrogels have been synthesized, which are non-adhesive, elastic and displayed enhanced anti-microbial properties which were evaluated by disk diffusion test on *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The release of silver nanoparticles from these hydrogels followed fickian diffusion and antimicrobial activity against various bacteria was comparable to ampicillin. More recently Ag NPs have been used in treatment of wounds and burn injuries. Chronic wounds most often contain pressure ulcers which are a type of lesion over subcutaneous tissue caused by external pressure leading subsequently to tissue necrosis. PVP/alginate/chitosan hydrogels with Ag NPs were synthesized by in-situ crosslinking via gamma radiation and were found effective in treating pressure ulcers by demonstrating antimicrobial properties and absence of toxicity against cells. The hydrogels exhibited enhanced reduction

of bacterial cell growth and high cellular viability of adult human dermal fibroblasts. Thus, these nanocomposite hydrogels can provide a more price effective alternative to the conventional treatment strategies.

### Iron Oxide Nanoparticles

Iron oxide nanoparticles (IONPs) have been used to tailor properties of polymeric hydrogels. Higher concentration of IONPs formed more crosslinks between the nanoparticles and polymer leading to stiffer and tougher nanocomposite hydrogels. These can also provide enhanced electrical conductivity than smaller IONPs which displayed a lower crosslinked density. IONPs in conjugation with poly(vinyl alcohol) (PVA) have been utilized to design PVA-IONPs composite degradable hydrogel system. In this system IONPs aid in physical crosslinking which is achieved by low temperature thermal cycling (LTTC). Additionally, LTTC also aids in development of multifunctional degradable PVA hydrogels wherein it controls mechanical properties of the hydrogel to mimic soft tissue mechanics. There was a sustained drug release from the PVA-IONPs hydrogel composite over a period of 1 month in EDTA making it a suitable degradable drug carrier. Usage of IONPs not only ensures degradation products to be biocompatible, but IONPs can also be used as contrast enhancements in MR and CT imaging allowing tracking of drug location which is essential in treatment of liver cancer. The approach can be replicated in conditions which are more representative of the physiologic conditions in regards to the type, concentration and pH of the media to discern their behavior in our body. Recently there has been interest in stimuli responsive hydrogels which release their drug reservoir on response to certain mechanical cues. Stimuli-responsive injectable hydrogels were synthesized with magnetic nanoparticles in thermoresponsive polymer. For example, citric acid coated  $\text{Fe}_3\text{O}_4$  magnetic nanoparticles synthesized via *co*-precipitation method can be entrapped within poly(*N*-isopropylacrylamide-*co*-acrylamide) (poly(NIPAM-*co*-AM)) shell followed by loading with Doxorubicin (DOX). The drug loaded IONPs can then be incorporated within an injectable hydrogel for localized, sustained and temperature dependent release of the therapeutic.

### Alumina and Titania Nanoparticles

Alumina ( $\text{Al}_2\text{O}_3$ ) and titania ( $\text{TiO}_2$ ) nanoparticles have been incorporated in polymeric matrix to augment cellular migration and adhesion which improves bioactivity of the nanocomposites. Hydrogel composites with  $\text{TiO}_2$  nanosticks have been conceived for cartilage tissue engineering. The hydrogel consists of dual polymeric matrix of poly(vinyl alcohol) (PVA) and polyvinyl pyrrolidone (PVP) which provides elasticity, hydrophilicity, porosity and biomechanical properties similar to the cartilage tissue. The  $\text{TiO}_2$  nanosticks not only act as reinforcement, enhancing mechanical and tribological properties but also promotes cell migration, adhesion and proliferation. This features make these hydrogels suitable for cartilage tissue regeneration.

### Zinc Oxide and Copper Oxide Nanoparticles

Metal and metal oxide nanoparticle-based nanocomposites can also show improved antimicrobial properties. Carrageenan based hydrogels with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles respectively were prepared which displayed strong antibacterial efficacy against pathogenic bacteria such as *Escherichia coli* and *Listeria monocytogenes*. Additionally, hydrogels with ZnO nanoparticles exhibited higher mechanical, thermal and antimicrobial properties than hydrogels containing CuO nanoparticles. ZnO NPs have also been utilized to make hydrogels for wound dressing. Current synthetic or natural wound dressing materials lack mechanical strength, flexibility, blood clotting and antimicrobial properties with added limitation of non-biodegradability. The new hydrogel systems are based on poly vinyl alcohol (PVA), starch (St), and chitosan (Cs) compositions with ZnO NPs. The hydrogel matrix exhibits good biocompatibility, biodegradability, water binding capacity and antifungal property. The antimicrobial property of the hydrogel is due to the release of  $\text{Zn}^{2+}$  ions which destructs the lipids and proteins of the bacterial cell wall membrane exuding the cell contents, resulting in bacterial cell death. ZnO nanoparticles in addition to possessing antimicrobial properties promote migration of keratinocyte towards the wound site, promoting faster healing. The hydrogels loaded with ZnO NPs are expected to achieved faster wound healing as compared to control groups. The healing process can be further accelerated by loading drug in the hydrogel matrix and ensuring its sustained release.

### Carbon Nanomaterial Based Nanocomposite Hydrogels

Carbon nanomaterials exhibit distinct physiochemical properties due to their ability for surface modification,  $\pi$ - $\pi$  interactions, biocompatibility and high adsorption capacity. Many carbon-based nanomaterials such as carbon nanotubes (CNTs), nanodiamonds (NDs), and graphene oxide (GO), can be used to obtain multiple functionalities in the nanocomposite hydrogels such as electrical conductivity and enhanced mechanical properties. These functionalities make carbon nanomaterial-based nanocomposite hydrogels an attractive choice for applications in biosensors, actuators, cardiac biomedical devices and scaffolds for tissue engineering.

### Carbon Nanotubes (CNTs)

CNTs are categorized into single-walled or multi-walled CNTs. CNTs exhibit strong  $\pi$ - $\pi$  self-interactions manifesting hydrophobic nature which limits their interaction with the polymeric structure as it is hydrophilic. To bypass this limitation, the carbon-based

nanoparticles are functionalized with polar groups such as hydroxyls, amines and carboxyls which prevent formation of aggregates and expedite dispersion of the nanoparticles in the hydrophilic polymer. Single-walled CNTs (SWCNTs) are made of single rolled-up graphene sheets of carbon atoms whereas the multi-walled CNTs (MWCNTs) consist of concentric cylinders made of multiple layers of graphene sheets and have a higher structural organization. CNTs reinforced nanocomposites can be used to synthesize tissue constructs which are electrically conductive and will enable *in vitro* engineering of muscle, nerve and cardiac tissues. Additionally, CNT reinforcement enhances mechanical properties of the tissue scaffold by significantly improving its elastic modulus and exhibit higher porosity which is appropriate for ECM deposition, exchange of nutrients and waste metabolites. CNTs have the dimensionality to simulate the structure of triple-helix collagen fibrils which are present in the extracellular matrix of osteoblasts, enabling the CNTs to guide regeneration of bone tissue. There has been a reported increase in osteoblast viability and osteogenic activity compared to control in presence of both SWCNTs and MWCNTs. In addition, an increase in mineralized matrix and bone like nodules formation indicating that these nanoparticles are non-toxic at low concentrations and promote biomineralization.

MWCNTs have also been functionalized with chitosan to lower their toxicity and utilize it as a more efficient drug delivery vehicle. A thermosensitive PCL-PEG-PCL *co*-polymeric hydrogel was developed incorporating the chitosan functionalized MWCNTs and loading them with doxorubicin (Dox) for a near infrared (NIR) triggered drug release. The MWCNTs absorb NIR light and converts it to heat which disrupts the hydrogel network by a sol-gel transition and releases the drug. But as the NIR light is turned off, there is a structural recovery of the hydrogel and drug release will be restrained. Thus, the hydrogel system offers a controlled and sustained delivery of therapeutic, improving the drug efficacy.

Recent studies showed hydrogel made of poly-acryloyl-6-amino caproic acid (PAACA) and reinforced with CNTs display enhanced strength and self-healing properties. Crack damage induced to the nanocomposite healed, subsequently restoring the nanocomposite to its same original strength due to interactions of the amide groups in the side chain with the carboxyl group. But a faster healing is observed when aqueous solution of low pH is added to the damaged zone. This expedited healing is due to the polar groups of opposite side chains displaying hydrogen bonding in the nanocomposite hydrogel network along with suitable balance between the hydrophobic and hydrophilic interactions.

### **Nanodiamonds**

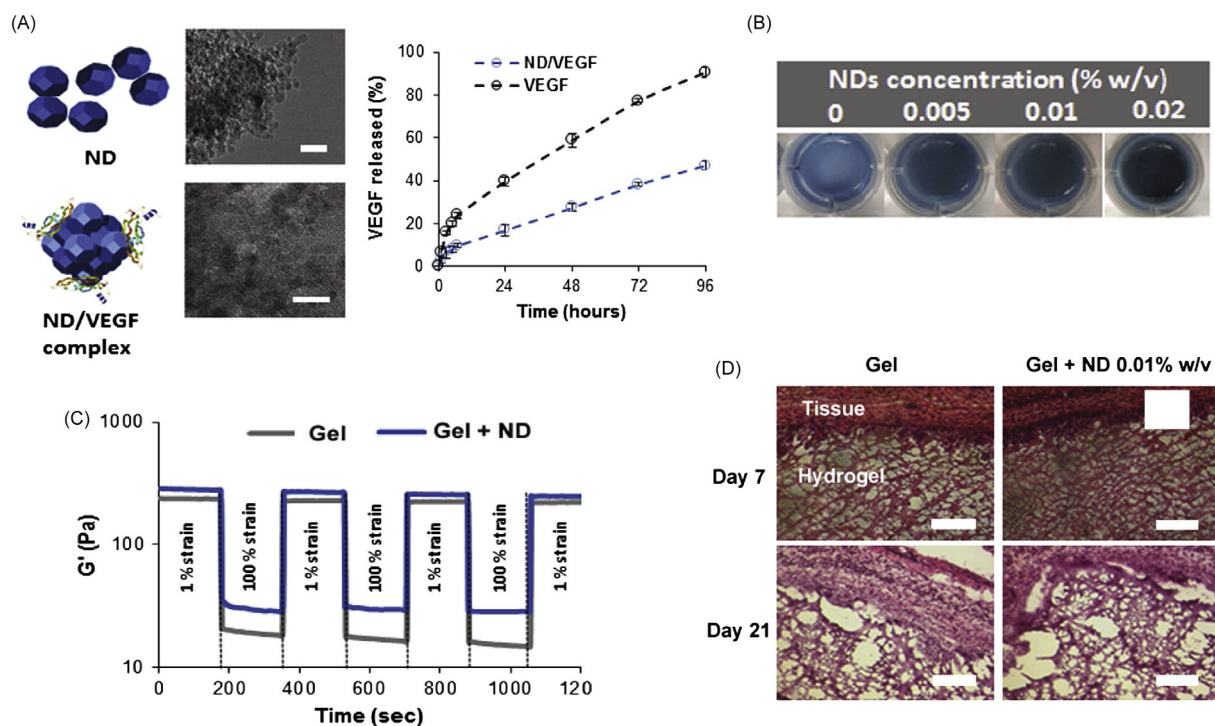
Nanodiamonds exhibit higher biocompatibility compared to other carbon materials and they have shown potential to deliver bioactive agents due to their higher surface area-to-volume ratio. In a recent study, nanodiamond based injectable chitosan-gelatin nanocomposite hydrogels have shown potential to be used for sustained release of vascular endothelial growth factor (VEGF). Functional groups on surface of nanodiamonds help in forming complexes with VEGF molecules. These complexes can be incorporated in hydrogels to control the release kinetics of the growth factor (Fig. 4A and B). The nanocomposites exhibit complete strain recoverability, aiding in making them injectable (Fig. 4C). As the nanocomposite was injected, it formed a local depot of the hydrogel at the site of injection and the nanodiamond modulated the release rate of VEGF, maintaining a steady state release. The *in vivo* studies also demonstrated biocompatibility and biodegradability of the nanocomposite hydrogels with minimum signs of inflammation (Fig. 4D). Additionally, there was also significant enhancement in mechanical properties of the injectable hydrogel without change in its thermosensitive gelation properties.

### **Graphene and Graphene Oxide**

There has also been substantial progress in using graphene and graphene oxide nanosheets for various biomedical applications. Surface functionalized graphene oxide sheets are covalently conjugated with polyacrylamide generating mechanically stiff hydrogels with excellent tensile strength and elastic modulus. These nanocomposite hydrogels can be utilized at locations which are under constant mechanical stress. Recently 3-dimensional (3D) ternary hydrogels consisting of graphene oxide (GO), hydroxyapatite (HA) nanoparticles and chitosan (CS) were developed by self-assembly, simultaneous reduction and cross linking of the components. These composites displayed high porosity, enhanced mechanical properties and good biocompatibility which makes them a suitable candidate for usage in biomaterials. Biocompatible conducting hydrogels with chitosan and lactic acid as matrix and graphene as reinforcement were developed using a facile technique of using reduced graphene oxide and lactic acid as crosslinker. The composite hydrogels exhibit enhancement in mechanical properties with increase in conductivity and significant rise in tensile strength while retaining processability which makes them robust and suitable for extrusion printing of scaffolds. The low graphene content is also useful in making the hydrogel composites suitable for cell seeding, proving them excellent candidates for tissue engineering scaffolds.

### **Inorganic Nanomaterial Based Nanocomposite Hydrogels**

There has been significant progress in the past decade to investigate effects of incorporating inorganic nanomaterials in natural and synthetic polymer networks for tissue engineering applications. Inorganic nanomaterials such as hydroxyapatite (HA), 2D nanosilicates, bioglasses and calcium phosphate nanoparticles are based on materials found in biological tissues such as bone and show significant bioactivity. Nanoparticles impart reinforcement and osteoconductivity while the polymers provide flexibility and



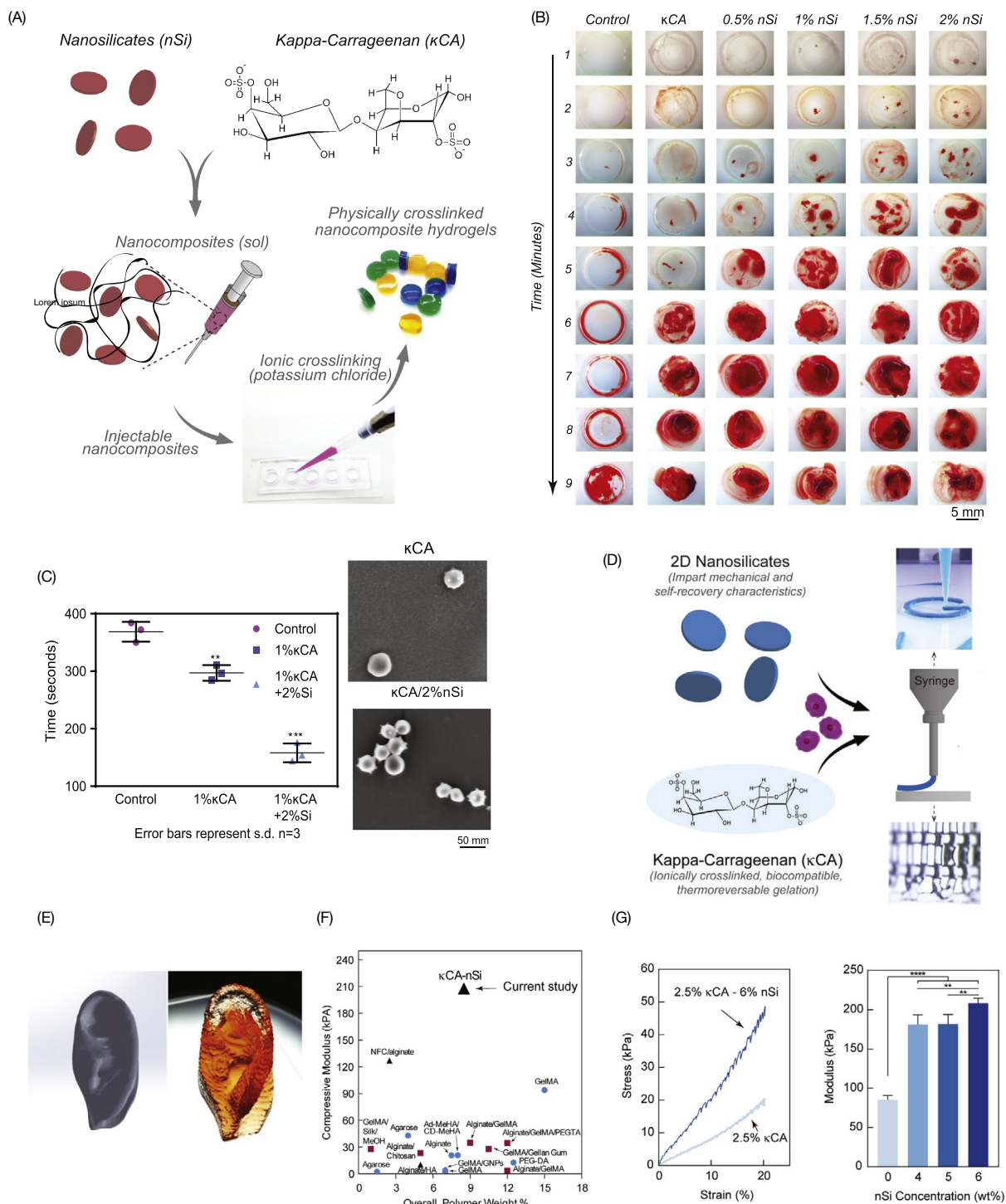
**Fig. 4** Nanodiamond (ND) based injectable nanocomposite hydrogel. (A) TEM images of ND and ND/VEGF complex exhibiting sustained release. (B) Injectable hydrogels with different concentrations of NDs. (C) Strain cycles demonstrating almost complete recoverability of injectable hydrogels. (D) Histological analysis with H&E staining of the explants revealing no infiltration of inflammatory cells. Adapted and reproduced by permission from Elsevier (Pacelli, S., Acosta, F., Chakravarti, A. R., Samanta, S. G., Whitlow, J., et al. (2017). Nanodiamond-based injectable hydrogel for sustained growth factor release: preparation, characterization and in vitro analysis. *Acta Biomaterialia* **58**, 479–491) © 2017.

stability to the nanocomposite structure. Surface chemistry favoring protein adsorption and enhanced matrix stiffness makes these nanocomposites an ideal choice for bone tissue engineering.

### Nanoclay and Nanosilicates

2D nanosilicates possess characteristic intrinsic and extrinsic properties like their surface charge, particle size and shape which have been proven to be beneficial towards stem cell differentiation and tissue regeneration. Nanosilicates induce human mesenchymal stem cells (hMSCs) towards osteogenic differentiation without any osteoinductive stimulators like bone morphogenic protein-2 (BMP-2), which makes them suitable candidates in designing hydrogels without any growth factors and osteoinductive supplement. The role of nanosilicates in hMSC differentiation and involvement of various cellular pathways in the process has also been thoroughly investigated. The osteoinductivity of nanosilicates when combined with synthetic poly (glycerol sebacate) (PGS) to configure hydrogels, exhibited improved cell adhesion, spreading, proliferation and osteogenic differentiation of preosteoblasts. The nanocomposites formed a microporous structure and with a minor addition of nanosilicates there was substantial increase in the value of compressive modulus along with accelerated degradation of the hydrogels. There was also enhanced protein adhesion on the hydrogel scaffolds with boost in matrix mineralization and ALP activity, directing preosteoblasts towards osteogenic differentiation. Thus, these nanocomposite hydrogels exhibit significant potential for use in bone tissue engineering applications.

The 2D clay nanosilicates possess layered structure with negative surface charge and positive edge charge. Given the negative surface charge, silicates exhibit great affinity for biomolecules and bind to them via electrostatic and Van der Waals' interactions. Due to this surface chemistry, they have been used for bone remodeling and delivery of therapeutics. Strontium ranelate (SRA), a drug for treatment of osteoporosis was encapsulated in poly(caprolactone) (PCL) nanocomposites reinforced with nanosilicates for osteogenic regeneration. SRA electrostatically interacts with nanosilicates, without interfering with its interlayer spacing. This interaction extends the dispersity of laponite in PCL by organo-modification and aids in the controlled release of SRA. Additionally, the human osteosarcoma cells can be successfully directed towards osteogenesis due to the dual osteoinductive effect of laponite and SRA. Recently, the surface chemistry of nanosilicates for wound healing applications was investigated (Fig. 5A). In addition to enhancing mechanical strength and protein release, nanosilicates incorporated in kappa carrageenan ( $\kappa$ CA) significantly reduces clotting time by activating the intrinsic pathway of hemostasis (Fig. 5B and C). Furthermore, when these nanocomposites are loaded with vascular endothelial growth factor (VEGF), a controlled release is observed thereby achieving complete wound healing in



**Fig. 5** Clay nanocomposite hydrogel scaffolds. (A) Synthesis of  $\kappa$ CA-nanosilicate injectable hydrogels for wound healing application. (B) Activated clotting time is reduced as nanosilicate content is increased in hydrogels. (C) Hydrogels with 2% nanosilicates are able to form clot by attachment and activation of platelets on the hydrogel surface. (D) Synthesis of thermoresponsive and shear thinning bioinks using nanosilicates and  $\kappa$ CA. (E) Printed structures with  $\kappa$ CA-nanosilicate bioinks. (F) Printed structures have higher modulus compared to currently available hydrogel-based bioinks. (G) Increasing silicate concentration demonstrated higher modulus and stiffness in printed structures. Adapted and reproduced by permission from Elsevier (Lokhande, G., Carrow, J. K., Thakur, T., Xavier, J. R., Parani, M., et al. (2018). Nanoengineered injectable hydrogels for wound healing application. *Acta Biomaterialia* **70**, 35–47) © 2018 and American Chemical Society (Wilson, S. A., Cross, L. M., Peak, C. W., and Gaharwar, A. K. (2017). Shear-thinning and thermo-reversible nanoengineered inks for 3D bioprinting. *ACS Applied Materials & Interfaces* **9**(50), 43449–43458) © 2017.



human umbilical vein endothelial cells (HUVECs) in vitro within 3 days. This demonstrates the silicate nanoparticles are capable of enhancing and bestowing new characteristics to polymer nanocomposites making them applicable in tissue regeneration.

Nanosilicates have also been used in the reinforcement of hydrogels to be employed as tissue scaffolds. Nanocomposite hydrogels were fabricated with nanosilicates incorporated in a silicate-hydroxypropylmethyl cellulose (Si-HPMC) scaffold for treating cartilage defects. The laponite nanoparticles due to their physiochemical properties are able to form an interpenetrating network and enhance mechanical strength, impeding gelation time of the Si-HPMC scaffold which is of prime significance for its application as injectable hydrogel in regenerative medicine. Laponite-driven nano-reinforcement of the hydrogel shows no significant effect on oxygen diffusion, cell viability or extracellular matrix formation, thus highlighting its relevance in articular cartilage regeneration. The significance of nanosilicates in 3D printing has also been demonstrated successfully (Fig. 5D). Incorporation of silicate nanoparticles in polymer hydrogels imparts shear thinning and thixotropic characteristics to the nanocomposite hydrogels enabling them to be successfully used for printing of three-dimensional tissue scaffolds (Fig. 5E). Viscosity of the nanocomposites was found to increase with increasing silicate content, reaching levels sufficient to be used as ink for printing. It was also demonstrated that tuning the polymer-nanosilicate ratios provides a high modulus and stiffness to the printed constructs (Fig. 5F and G). Additionally, the nanosilicates allows modulation of thermo-reversible gelation of the nanocomposites, facilitating printing tissue constructs with high shape retention.

### Calcium Phosphate and Hydroxyapatite Nanoparticles

Calcium phosphate is a naturally occurring bone mineral present in its carbonated apatite form in the bone tissue. Nanomaterials based on calcium phosphate are osteoinductive and widely used as bone substitutes. Hydroxyapatite is an inorganic component of the bone tissue and is often used as reinforcement of nanocomposite hydrogel scaffolds designed for bone tissue engineering. 3D scaffolds made of polyacrylamide grafted cellulose and hydroxyapatite exhibited comparable mechanical and structural properties as the trabecular bone. It was also observed that varying hydroxyapatite concentration enabled tuning of porosity which regulated the structural integrity of the scaffold. The nanocomposites at low porosity were able to mimic the morphological properties of cancellous bone due to the presence of interconnected pores, large surface area and cell aggregation. The similar reinforcing property of hydroxyapatite was demonstrated by nanohydroxyapatite (nHAP)/collagen nanocomposite hydrogels for use as synthetic bone graft substitutes. nHAP embedded in the collagen matrix buttressed the nanocomposite structure by increasing its compressive strength. nHAP embedding improves the load bearing capacity of the nanocomposite scaffolds and also creates bioactive sites for protein adhesion, thus enhancing cell attachment. nHAP have been embedded in gelatin matrix to provide bioactive characteristics that can be used for bone tissue engineering.

### Bioactive Glass Nanoparticles

A drawback associated with hydroxyapatite nanoparticles is excessive nucleation during their fabrication which interferes with its bioactivity. As a solution, hydroxyapatite have been replaced with bioglasses in various synthetic and natural polymers for stronger architecture and enhanced bioactivity. Bioglass reinforced into the polymer network improves mechanical properties of the nanocomposite hydrogels and strengthens the microstructure by almost doubling the compressive modulus. Furthermore, It was also discerned that bioglass incorporated in poly(lactic acid) (PLLA) enables neutralization of the by-products of degradation thus reducing the side-effects of nanocomposite wear. The degradation profile of the nanocomposites shows a decrease in pH compared to unmodified PLLA. Bioglass integration has also shown to increase apatite formation in nanocomposites thus imparting mineralization potential to the scaffolds.

Reinforcement of the nanocomposite architecture is one the primary characteristic of inorganic nanoparticles. Incorporation of these nanoparticles in polymer networks is widely applied in directing cells towards osteogenic lineage. Their interaction with polymers is greatly influenced by preparation methods, network type, degradation kinetics and content, thus providing a tunable range of nanocomposites for bone tissue engineering.

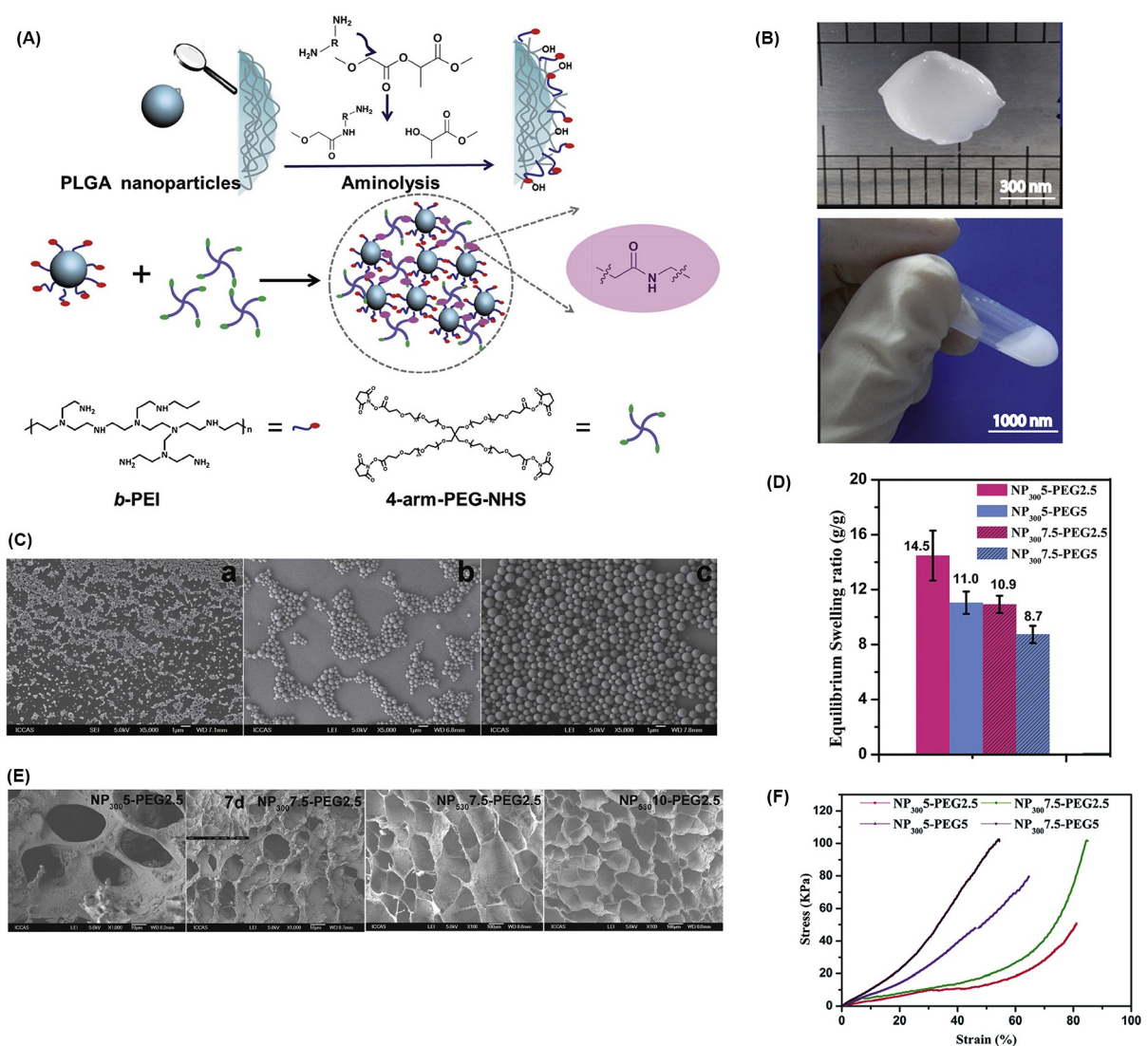
### Organic Nanomaterial Based Nanocomposite Hydrogels

Organic nanomaterials are natural or synthetic polymer moieties with extensive applications in drug delivery and cancer immunotherapy. Dendrimers, liposomes, hyperbranched polymeric nanoparticles and micelle are some types of polymeric nanoparticles used for varied applications in tissue engineering. Various parameters of polymeric nanoparticles can be tuned to induce an immune response against cancerous cell growth. Particle size and shape are the most widely studied and reported characteristics as they affect blood circulation time, cellular uptake, antigen presentation and T cell immunity. Recently poly(vinyl alcohol) (PVA) embedded with polystyrene nanoparticles of varying shapes and sizes were synthesized to show immunomodulation. In vivo studies of the nanocomposite hydrogels revealed that varying the shape of the nanoparticles presented variation in the immune response wherein smaller sized nanoparticles showed better immune response compared to larger size. This was attributed to the surface characteristics of polymeric nanomaterials.

## Hyperbranched Nanoparticles and Dendrimers

Hyperbranched nanoparticles and dendrimers have shown great promise in the reinforcement of tissue scaffolds *in vitro*. For example, poly(lactic acid-glycolic acid) (PLGA) nanoparticles can be crosslinked to 4-arm poly(ethylene glycol) to obtain stiffer nanocomposite hydrogels (Fig. 6A and B). Tuning the concentration of the nanoparticles (Fig. 6C) allows for change in strength, porosity (Fig. 6D and E), modulus (Fig. 6F) and viscoelasticity of the nanocomposite hydrogels. The homogenous distribution of the hyperbranched polymeric nanoparticles through the nanocomposite structure further adds to their reinforcement attribute. Also poly(2-hydroxyethyl methacrylate) (PHEMA) nanoparticles dispersed through fibrin gels display enhanced viscoelasticity and microstructure reinforcement due to peptide bond formation between carboxylic end group of nanoparticles and the amine end group of the fibrin polymer resulting in preservation of the natural crosslinking.

Dendritic nanoparticles have also been investigated immensely for their 3D branched, interconnected and highly porous morphology which helps in improving the mechanical and surface attributes of the nanocomposite hydrogel in which they are integrated. Their hyperbranched structure and globular architecture allows for affiliation for both hydrophobic and hydrophilic moieties. This enables a spatiotemporal control over drug delivery and sustained dosage for improved therapeutic efficacy. Surface modification of poly(L-lactic acid) (PLLA) scaffolds with polypropylene imine dendrimer results in a more hydrophilic nanocomposite hydrogel with neutralization of degradation products of PLLA. Interestingly, the dendrimers formed interpenetrating network



**Fig. 6** Nanocomposite hydrogel scaffolds with controlled porosity and mechanical enhancement. (A) Fabrication of branched PLGA nanoparticle/4-arm-PEG hybrid nanocomposite hydrogels. (B) Nanocomposite hydrogels with various PLGA nanoparticles. (C) SEM images of PLGA nanoparticles formed by microfluidics (a) and premix membrane emulsification (b, c) techniques. (D) Equilibrium swelling ratios of different nanocomposite hydrogels. (E) SEM characterization displaying controlled porosity in different nanocomposite hydrogels. (F) Mechanical characterization of different nanocomposites showing significant mechanical enhancement. Adapted and reproduced by permission from Royal Society of Chemistry (Zhuang, Y., Shen, H., Yang, F., Wang, X., Wu, D. (2016). Synthesis and characterization of PLGA nanoparticle/4-arm-PEG hybrid hydrogels with controlled porous structures. *RSC Advances* 6(59), 53804–53812) © 2016.

with PLLA via electrostatic interactions and amine bonds thus reducing degradation rate and buffering the pH of the degradation environment. PLGA nanoparticles have also been utilized to engineer nanocomposites with controlled porosity and mechanical properties which can be potentially used for tissue engineering scaffolds. Similar results of reduced degradation rate and increased biocompatibility were also reported for poly(amidoamine) (PAMAM) incorporated through 3D gelatin matrix. The dendrimers enhanced water absorption capacity and protein adsorption of the nanocomposite as the free amine group in PAMAM interacted with water and protein molecules respectively, enhancing the nanocomposite elasticity.

### Liposomes and Micelles

Liposomes nanoparticles form nanocomposites with natural and synthetic polymers which can be used as delivery vehicles for therapeutics. Liposomes show smooth conformational transitions for binding with hydrophilic molecules. Liposomes were combined with PLGA particles in a core-shell form for the sustained delivery of IL-2 and TNF- $\beta$  for enhanced tumor immunotherapy. Micelles are block amphiphilic copolymers with a size between 10 and 100 nm. There have been significant applications of micelles in cancer therapies as they exhibit excellent attributes for drug delivery and reactivating native immune response. Their small size allows for high drug loading and enhanced EPR effect with deeper tumor penetration, allowing for better tumor targeting. Micelles have a high drug loading capacity due to hydrophilic and hydrophobic moieties in their structure. Overall, polymeric nanocomposite hydrogels have paved their way as suitable candidates for tissue engineering, drug delivery and cancer immunotherapy as a result of their tunable structural and conformational attributes.

### Emerging Trends and Future Outlook

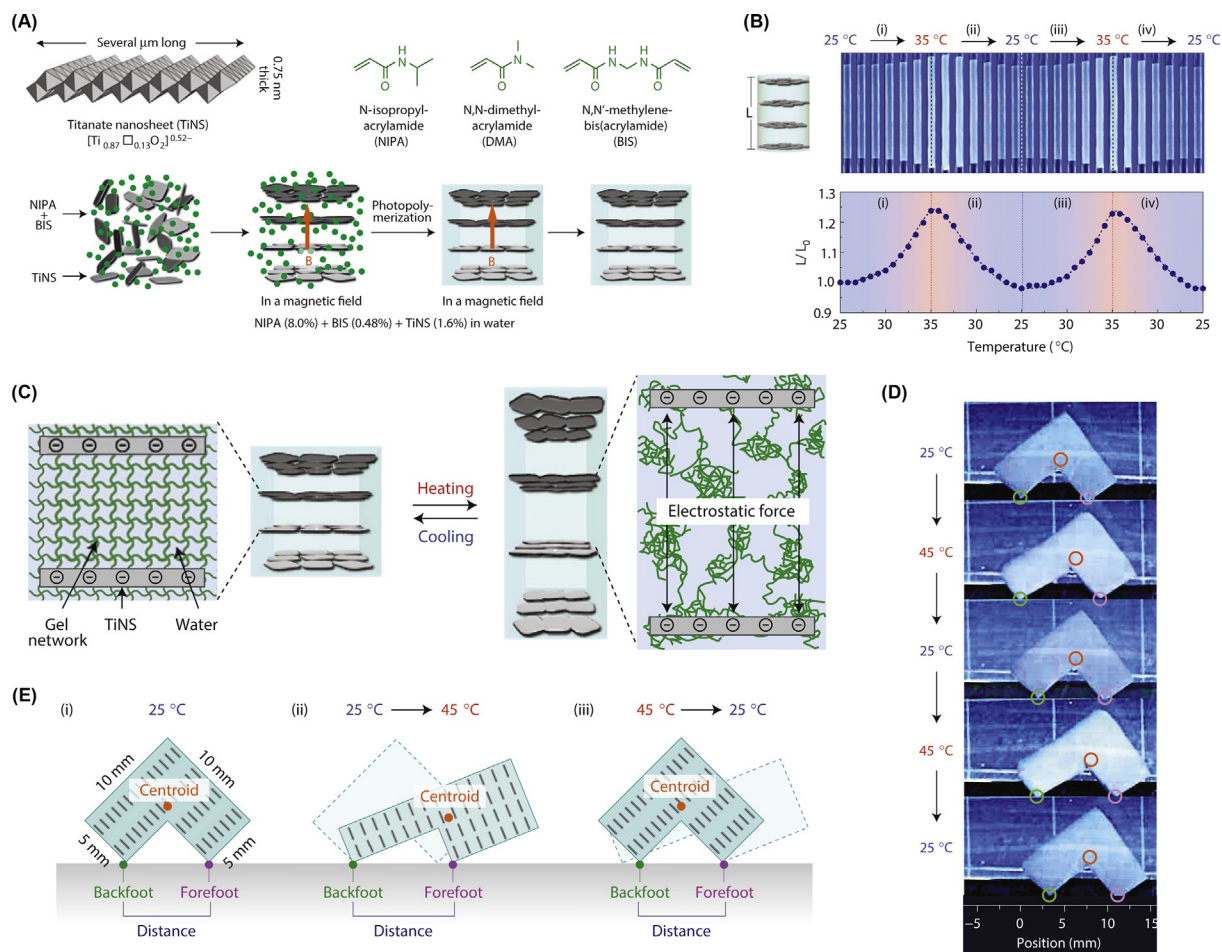
Recent progress in nanocomposite hydrogels is in the direction of incorporating biomimetic design in its composition. A significant progress in this direction has been designing hydrogels with highly ordered structure. Many soft tissues in our body such as blood vessels, cartilage, muscles and corneas are hydrogels exhibiting superior mechanical performance due to their ordered hierarchical structures.

### Ordered Nanocomposite Hydrogels

A common method towards fabricating highly ordered nanocomposite hydrogels is by utilizing magnetic and electric field. Magnetic hydrogels were synthesized by assembling magnetic Fe<sub>3</sub>O<sub>4</sub> nanospheres in acrylamide monomer. Due to the magnetic field the nanospheres form chain-like assemblies which are immobilized in the hydrogel with a thermally induced gelation. The ordered nanocomposite hydrogels display enhanced magnetothermal behavior which can be modulated by tuning the direction and intensity of the magnetic field. Doxorubicin (DOX) release from the nanocomposite hydrogels is also dependent on the directionality of the magnetic field. These nanocomposite hydrogels will be suitable candidates to design multifaceted stimuli responsive platforms for drug delivery, hyperthermal therapy and tissue engineering. Similar strategy can be employed with electric field to obtain highly ordered structures. Nanomaterials with permanent dipole moment can be aligned in a particular direction by an electric field. Methacrylated gelatin (GelMA) reinforced with carbon nanotubes (CNTs) and the CNTs were aligned in axial direction by tuning frequency and voltage of the electric current, to conceive nanocomposite hydrogels with strengthened mechanical properties and enhanced anisotropic electrical conductivity. The aligned CNT-GelMA nanocomposite hydrogels also allowed better tuning of electrical stimulation to skeletal muscle cells controlling their differentiation, which can be a useful tool for monitoring metabolism and glucose consumption in the body. This makes these nanocomposite hydrogel systems an efficient tool to design bio actuators biomarkers.

### Soft Robotics and Biosensors

Another emerging area of nanocomposite hydrogel application is in soft robotics and biosensing. Soft robots are flexible devices which execute programmable complex motions controlled by external stimuli. Soft robots can process and convert diverse stimuli such as light, temperature, humidity, volume change to concise mechanical movement or macroscopic actuation without structural failure. A thermoresponsive soft robot based on Poly(*N*-isopropylacrylamide) (PNIPAAm)/titanate(IV) nanosheets (TiNS) nanocomposite hydrogel was fabricated to possess a layered structured with TiNS being cofacially oriented due to presence of magnetic field (Fig. 7A and B). The soft robot was in a shape of bipedal walking object. When temperature was increased from 25°C to 45°C the backfoot of the of the soft robot elongated propelling it forward by tilting the centroid towards the front. Similarly, cooling it shortens the backfoot pulling it backwards but the corresponding centroid shift suppresses this motion (Fig. 7C and D). Thus, a repeated thermal cycling allows a unidirectional motion of the soft robot by mechanism of anisotropic energy conversion (Fig. 7E). Biosensors in contrast to soft actuators are capable of converting mechanical energy into other forms such as optical or electrical signals. A PNIPAM/rGO based nanocomposite hydrogel with colloidal silica nanoparticles was synthesized as a structural color material with NIR responsive properties. The hydrogel exhibited NIR dependent reversible bending behavior with color indication. The bioinspired structural color design can also be utilized in intelligent sensors and anti-counterfeiting barcode designs.



**Fig. 7** Nanocomposite hydrogels as functional soft robots. (A) Fabrication of ordered Titanate nanosheet (TiNS) reinforced nanocomposite hydrogel. (B) Synchronous thermoresponsive change in shape of nanocomposite hydrogel. (C) Thermoresponsive deformation of nanocomposite hydrogel driven by hydration and dehydration of hydrogel network. (D) Thermoresponsive processing profiles of the soft actuator. (E) Functioning mechanism of the soft actuator. Adapted and reproduced by permission from Springer Nature (Kim, Y. S., Liu, M., Ishida, Y., Ebina, Y., Osada, M., et al. (2015). Thermoresponsive actuation enabled by permittivity switching in an electrostatically anisotropic hydrogel. *Nature Materials* **14**(10), 1002) © 2015.

### Programmable Materials

The field of nanocomposite hydrogels has also grown to include fabrication of smart programmable hydrogels. These hydrogels are able to change their function sequentially, periodically or in a programmed fashion with certain stimuli. Recently self-assembled DNA quantum dot hydrogels that exhibit spectral tunability are fabricated. With guided DNA interactions these hydrogels displayed multi-functional properties such as specific cell targeting and enzyme linked drug delivery. The quantum dot DNA hydrogels were utilized for delivery of doxorubicin and exhibited increased potency against cancer cells. By functionalizing the quantum dots with aptamers, they were employed for programmed specific cell targeting and modulating protein expression through SiRNA delivery. A poly(acrylic acid) based hydrogel with tannic acid-coated cellulose nanocrystal reinforcement was synthesized to exhibit self-healing and self-adhesive behaviour. The dynamic reversible coordination interactions in the hydrogel endows it with excellent programmable electrical self-healing and strain sensitivity which can be utilized in designing strain sensors to monitor macro joint motions and micro responses such as pulse and heartbeat.

### Conclusion

Nanocomposite hydrogels show potential to be used in variety of different biomedical applications such as tissue engineering, therapeutic delivery, stem cell modulation and medical devices. These different biomedical and biotechnological applications are possible due to tunable nature of the nanocomposite hydrogels. The improved properties of the nanocomposite hydrogels are

mainly attributed to enhanced interactions between the polymer chains and the nanomaterials. Next generation of nanocomposite hydrogels will not only enhance mechanical and physical properties but will be able to deliver special bioactive cues which would make them more responsive and adaptable to the environment in which they would be utilized.

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