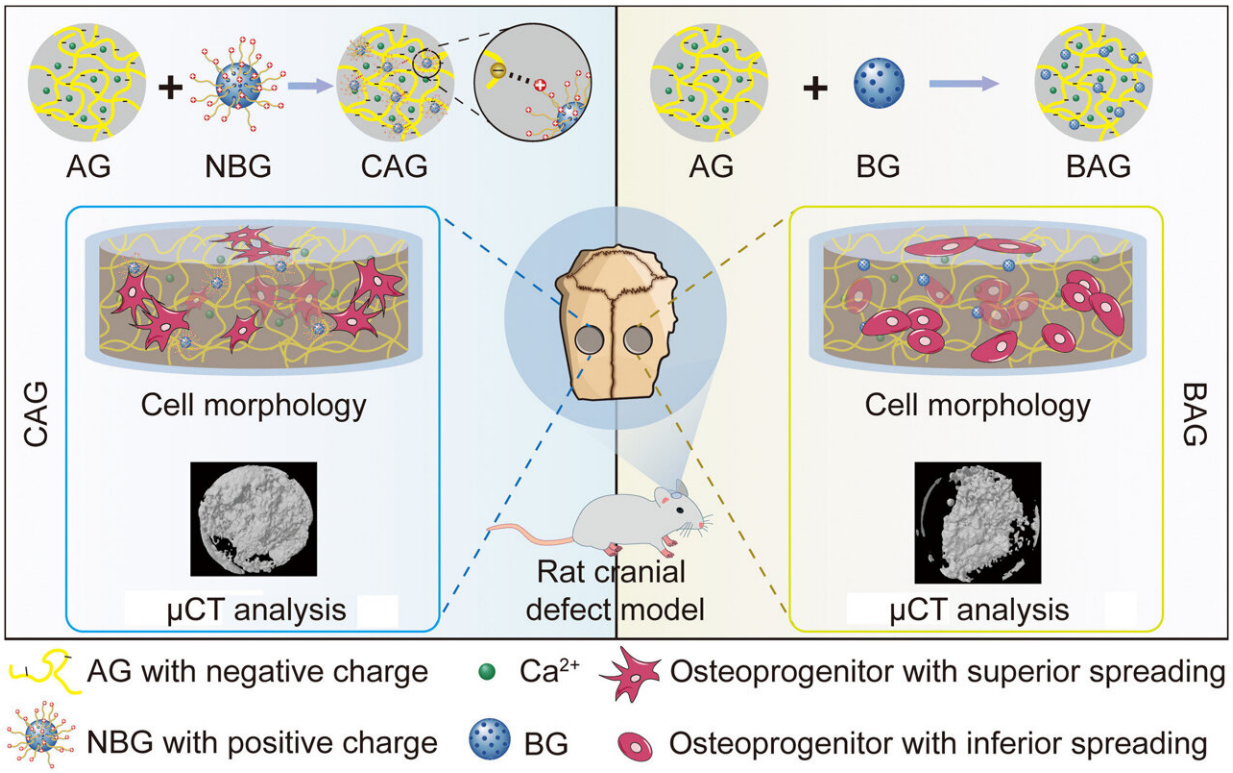
A biopolymer hydrogel with amino-functionalized bioactive glass for accelerated bone regeneration

December 17 2021, by Thamarasee Jeewandara



Preparation and biomedical application of hydrogels.CAG was fabricated by forming electrostatic interaction between cationic NBG and anionic AG. BAG was obtained by simply mixing AG and BG component. A rat cranial bone defect model was used to detect the biocompatibility and osteogenic activity of hydrogels. Cells in CAG exhibited favorable cell spreading, while there was no obvious cell pseudopodium observed in BAG. Moreover, bone regeneration was also enhanced in CAG compared with BAG. micro–computed tomography

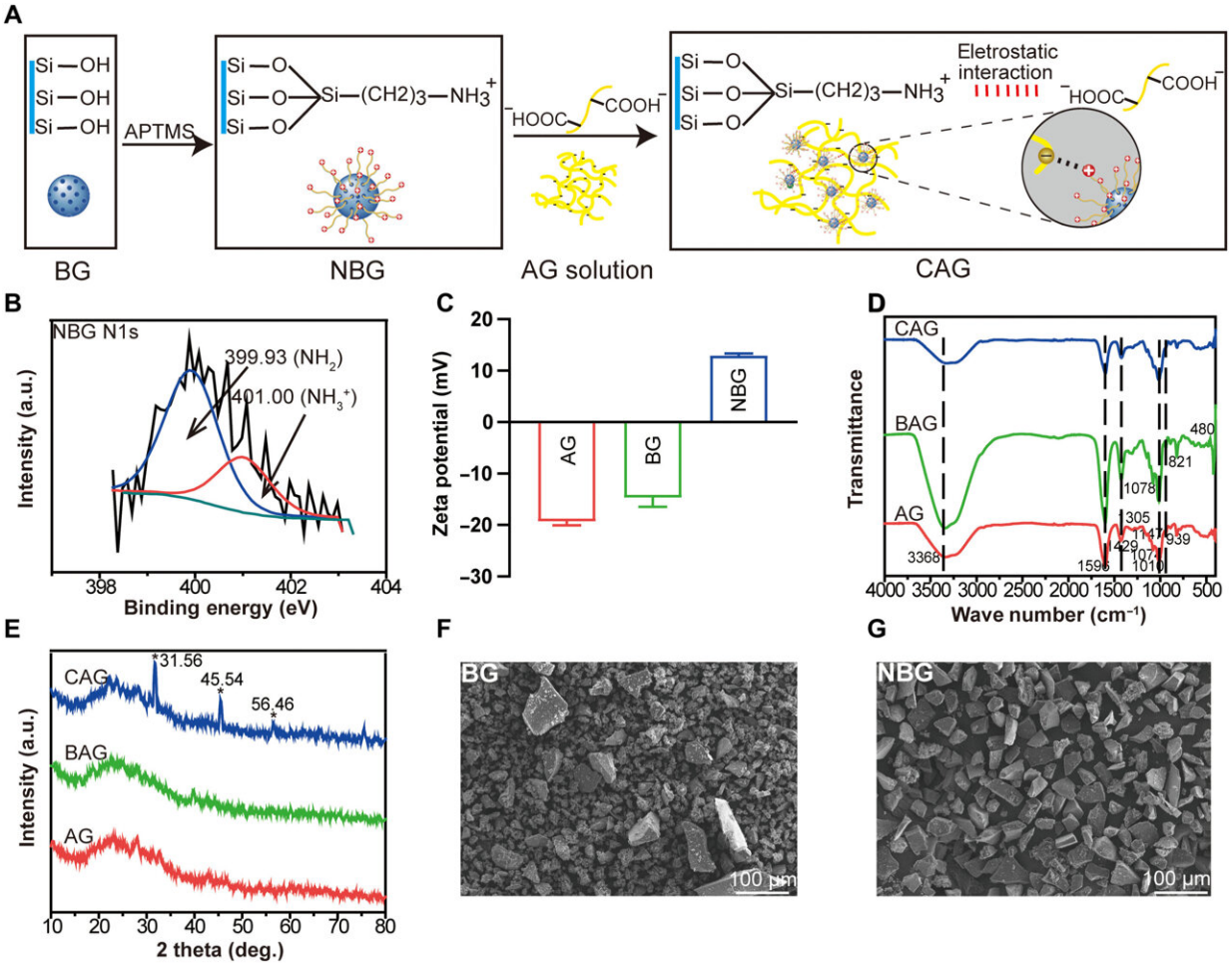
(μCT). Credit: Science Advances, 10.1126/sciadv.abj7857

Composite hydrogels can incorporate natural polymers and bioactive glass as promising materials for bone regeneration. However, the applications of such constructs are limited by poor compatibility between organic and inorganic phases. In a new study now published in *Science Advances*, Xinxin Ding, and a research team in medicine, in Shanghai China, formed an electrostatically reinforced hydrogel (abbreviated CAG) with improved interfacial compatibility. To accomplish this, they introduced amino-functionalized bioactive glass to the alginate/gellan gum matrix. When compared with bioactive glass, the electrostatically reinforced hydrogel indicated a more uniform porous structure with a pore size of 200 μm and an optimal compressive strength of 66 kPa. Using the reinforced hydrogel, the team promoted the phenotype transition of macrophages and upregulated the osteogenic gene expression of stem cells. They showed how new bone formation was also accelerated in vivo with enhanced biomineralization of the electrostatically reinforced hydrogel, with biocompatibility ideally suited for bone regeneration.

Bone regeneration in the clinic

In this study, Ding et al. developed hybrid hydrogels by establishing interfacial bonds between different phases. Bone defects typically occur in patients with severe trauma, osteitis and other bone deformities that are increasingly costly to repair. Methods of efficient bone repair are still a challenge for clinicians, therefore advances in materials science have led to the development of new biomaterials to enhance methods of bone regeneration. Researchers have developed natural biopolymer-based hydrogels with outstanding biocompatibility and biodegradability. Among the various natural polymers, alginate and gellan gum exhibit

potential for clinical applications, since they are economically feasible and easily gelled using calcium ions. Hydrogels made of gellan gum can also induce satisfactory osteogenesis with porous structural similarity to natural bone tissue for cell attachment and osteogenic differentiation. Despite existing methods, the mechanical properties of pure hydrogels are insufficient for bone defect repair in vivo. Since it is complex to synthesize hybrids with covalent interfacial bonds between different phases, non-covalent crosslinks are a more practical method for clinical applications.

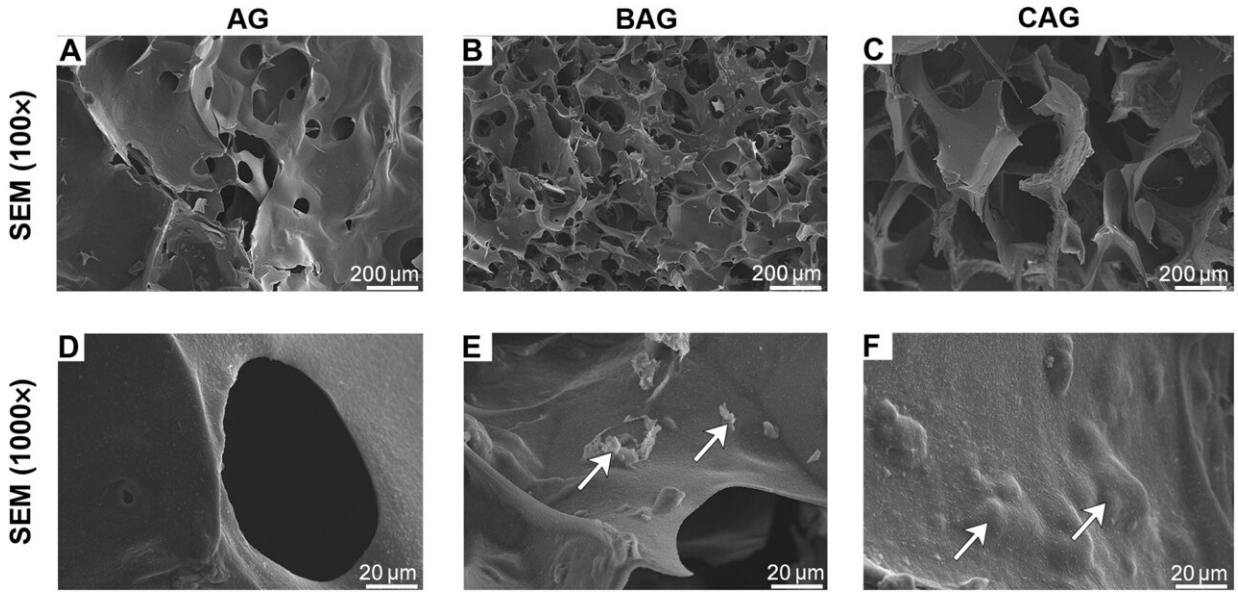


Structural analysis of synthesized hydrogels. (A) Scheme of the preparation of NBG and CAG. APTMS, (3-aminopropyl) trimethoxysilane. (B) High-resolution

x-ray photoelectron spectroscopy spectra of N1s in NBG particles. a.u., arbitrary units. (C) Zeta potential of AG, BG, and NBG. (D) Fourier transform infrared spectra of AG, BAG, and CAG. (E) X-ray diffractometer patterns of AG, BAG, and CAG. (F) Scanning electron microscopy (SEM) image of BG. (G) SEM image of synthesized NBG. Credit: Science Advances, 10.1126/sciadv.abj7857

Developing a novel hybrid hydrogel

The team developed an electrostatically reinforced hydrogel made of cationic amino-modified bioactive glass (NBG) and anionic gellan gum (AG). They improved the interfacial compatibility by forming electrostatic interactions between organic and inorganic phases. To improve the gelation network of the hydrogels, Ding et al. used calcium ions for ionic cross-linking. As a control, Ding et al. developed a type of bioactive glass (BAG) by mixing nonfunctionalized bioactive glass (BG) and gellan gum (AG). The researchers aimed to create a self-assembled gelation network of hydrogels with enhanced shear-thinning and self-healing ability. They aimed to form the hydrogels with electrostatic interactions between the constituent composites, characterize the physicochemical properties of hydrogels and investigate the bone regenerative capacity of diverse hydrogels. The team prepared the NBG (amino-modified bioactive glass) and CAG (electrostatically reinforced hydrogel) by physically mixing the components, followed by X-ray photoelectron spectroscopy and Zeta potential test to investigate electrostatic interactions in the hydrogels and identified grafted amino groups on NBG.



Cross-sectional images of different hydrogels using SEM analysis. (A to C) SEM images of AG, BAG, and CAG. (D to F) Magnified images of AG, BAG, and CAG. White arrows in (E) and (F) indicate the BG and NBG particles on the hydrogel surface. Credit: Science Advances, 10.1126/sciadv.abj7857

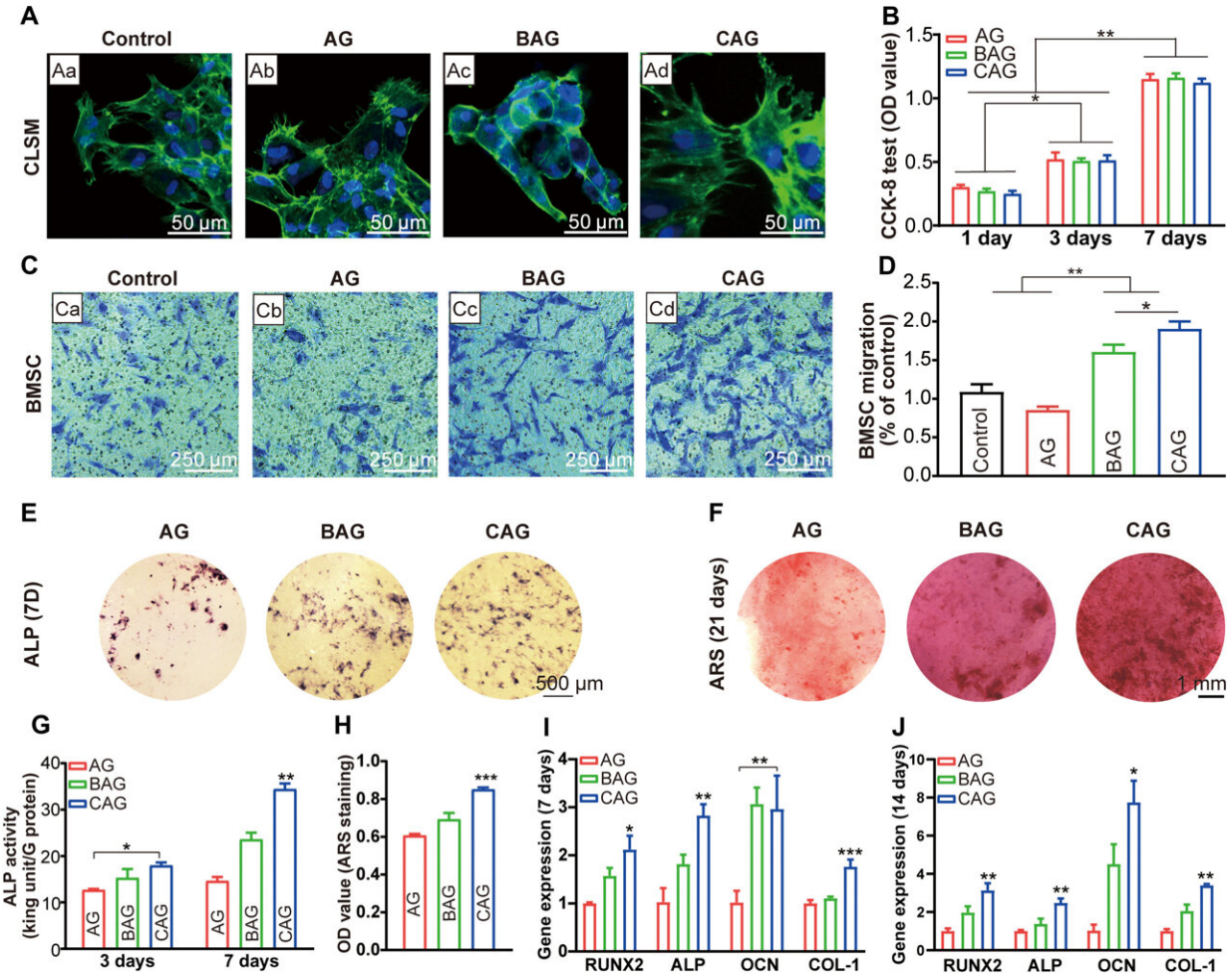
Analyzing the structure of hydrogels

Using representative Fourier transform infrared (FTIR) spectra, the team then analyzed the three types of materials. Using X-ray diffractometers, they understood and detected the potential crystalline phases in hydrogels. Ding et al. next studied the effect of particles on the hydrogel microstructure, and detected the morphologies of bioactive glass (BG) and amino-modified (NBG) bioactive glass via scanning electron microscopy. The scientists credited the presence of homogenous NBG particles to the synthetic method and subsequently analyzed the microstructure morphology of hydrogels by detecting cross-sectional images using SEM, where the porous surfaces of gellan gum (AG) were smooth. Meanwhile, incorporating inorganic particles substantially

affected the structural morphology of the biomaterials. The team also noted the identical three-dimensional (3D) porous architecture of BAG (the novel type of bioactive glass control) and CAG (the electrostatically reinforced hydrogel).

Physicochemical properties of diverse hydrogels

Since hydrogels are known for their property of swelling; one of the most important physical characteristics of the material, swollen hydrogels promoted the exchange of substances by absorbing nutrients from the external environment. By incorporating bioactive glass in this work, Ding et al. significantly decreased the swelling ratio of BAG (the novel bioactive glass) when compared with gellan gum. The fluid retention properties of the material allowed the infiltration of cells into scaffolds, and facilitated efficient nutrient transport between the extracellular matrix and the hydrogel. Upon in vivo implantation, the encapsulated cells were able to grow well in a nutrient rich environment. However, since hydrogels typically degrade after implantation in the human body, where scaffolds can degrade at a rate comparable with tissue regeneration. The scientists studied the degradation behavior of hydrogels by detecting the change in gel weight in time. The stable degradation rate of the CAG (electrostatically reinforced hydrogel) biomaterial allowed its retention in the osteogenic space for a long time after implantation with benefits for osteogenesis. The researchers additionally investigated the mechanical properties of hydrogels via rheology and compressive strength experiments.

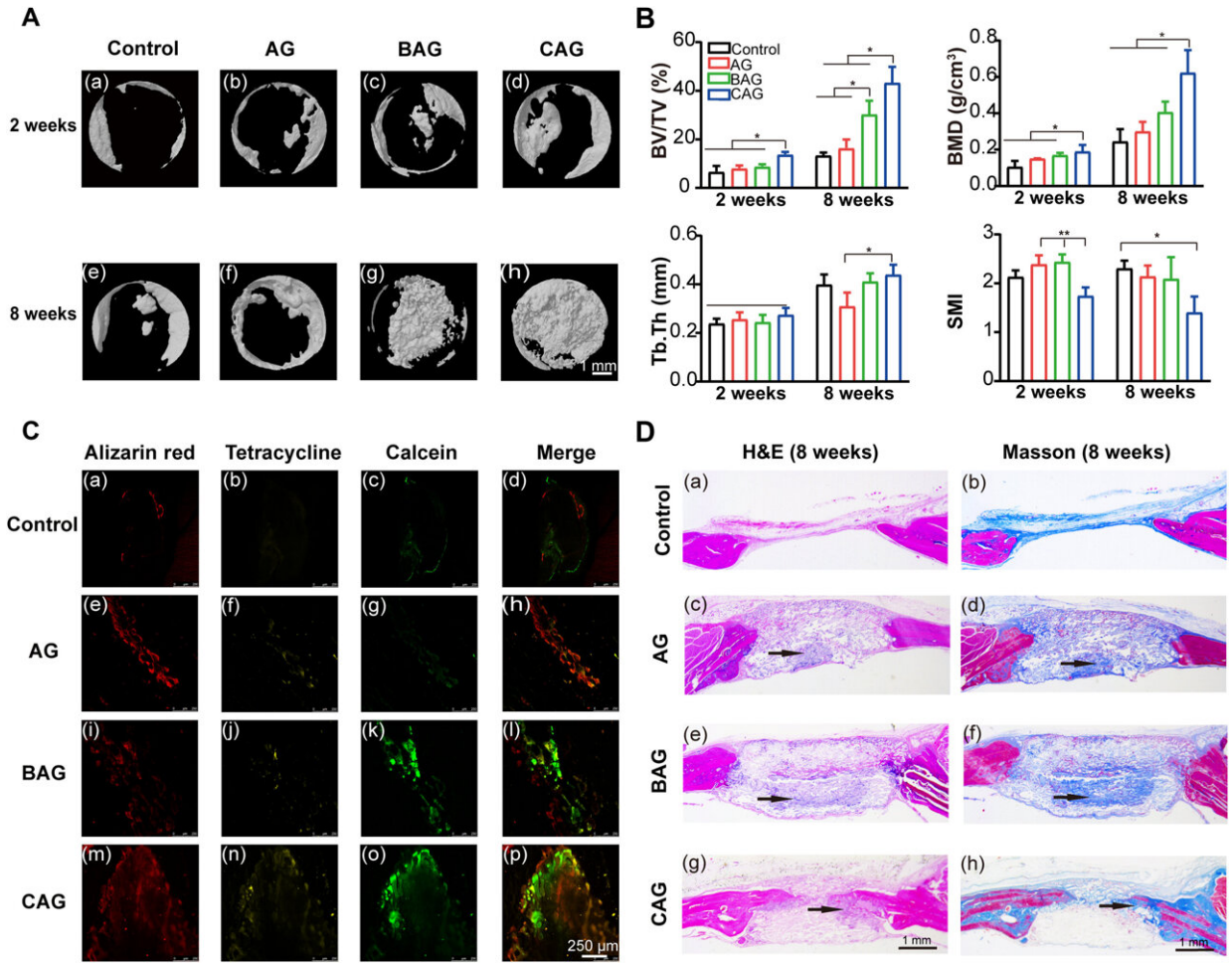


Osteogenic differentiation of BMSCs cultured on hydrogels. (A) Confocal laser scanning microscopy (CLSM) images of cells cultured for 24 hours [(a), control, (b) AG, (c) BAG, and (d) CAG]. (B) Cell Counting kit-8 (CCK-8) assay of cells cultured for 1, 3, and 7 days. **P

Immune response and osteogenic differentiation of the cells on the biomaterials in vitro

Materials scientists carefully monitor most new bone substitute materials for immune responses at the defect site. Here, Ding et al. investigated the cell migration ability and inflammatory gene expression to determine the immune response after hydrogel treatment. Both BAG and CAG biomaterials showed improved cell migration when compared to other controls and indicated a

successful induction of immune responses. To then understand cell biocompatibility, the team satisfied the parameters of cell morphology and migration on hydrogels for early tissue regeneration. To accomplish this, they used rat bone marrow stem cells and cultured them on the biomaterials. The CAG biomaterial assisted cell attachment and spreading, while amino groups on the NBG surface enabled protein adsorption for cell migration, while BAG did not show suitability for cell adhesion.



Newly bone formation in a rat cranial bone defect model. (A) μCT images of calvaria samples, (A, a to d) samples from 2-week post-surgery group. (A, e to h) Samples from 8-week post-surgery group. Images of (A) (a to h) exhibit the same scale bar of 1 mm. (B) Quantitative analysis of newly bone formation at 2 and 8 weeks. Notes: Bone volume/tissue volume (BV/TV), bone marrow density (BMD), trabecular thickness (Tb.Th), and structure model index (SMI). *P

Outlook—bone forming potential of hydrogels in vivo

Given the enhanced osteogenic performance of CAG, the team next investigated the material in a rat cranial defect model to detect bone regenerative capacity of hydrogels in vivo. They noted an inflammatory response upon implantation, which led to the recruitment of a large number of macrophages at the defect site. Ding et al. also noted the increased expression of bone marrow stem cells at the defect site, using immunohistochemistry and credited the enhanced recruitment of cells to the morphology of CAG, which contained relatively larger pores to facilitate cell recruitment and bone growth at the defect site, in order to accelerate bone repair. Further studies included micro-computed tomography methods and histology staining, alongside sequential fluorescence labeling to understand bone regeneration among diverse biomaterials. In this way, Xinxin Ding and colleagues showed how improved interfacial biocompatibility between different phases contributed to the improvement of composite hydrogels. The diverse biomaterials enhanced the capacity for bone regeneration and provided a convenient approach to develop new composite hydrogels that can be translated for clinical applications.

More information: Xinxin Ding et al, A biopolymer hydrogel electrostatically reinforced by amino-functionalized bioactive glass for accelerated bone regeneration, *Science Advances* (2021). DOI: 10.1126/sciadv.abj7857

Nathaniel Huebsch et al, Matrix elasticity of void-forming hydrogels controls transplanted-stem-cell-mediated bone formation, *Nature Materials* (2015). DOI: 10.1038/nmat4407