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Smart On-Demand and Programmed Injectable Gels for Cancer Treatment

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Hydrogels are often exploited biological materials in the transfer of biomolecules such as medications, DNA, and proteins owing to apparent features, including cytocompatibility and likeness to genuine human tissues. A number of biomaterials have the capacity to be injected, which allows for minimum invasion and eliminates the necessity of surgery to transplant pre-formed materials. This material is injected through the target location in a solution condition before gelling. If a stimulus generates gelation by the interaction of one or more stimuli, it can do so spontaneously. When such occurs, the material/system is referred to be stimuli-responsive since it reacts to its environment. In this context, we discussed the many triggers leading to gelation and reviewed the various processes by which the solution becomes a gel. We also reviewed several trials that used these gels to treat cancer. These materials show promise for treating cancer. Injectable hydrogels and stimuli-sensitive materials were the topics of several studies and reviews. To our knowledge, there has not been any research done on the use of smart injectable hydrogels for tumor therapy.

INTRODUCTION

Networks of hydrophilic polymers called hydrogels may absorb thousands of times their dry weight of water [1]. Because of their exceptional qualities, hydrogels are frequently used in medicinal applications [2]. Hydrogels, for instance, are typically biocompatible [3-6]. Its property is owing to the high-water content and resemblance to biological tissue in terms of chemical makeup and mechanical characteristics [7-10]. Controlling the kinetics of the processes is the main advantage of using hydrogels for medication delivery [11, 12]. To modulate the rate of medication entry through the surrounding tissues, for instance, the network porosity may be simply modified [13-15]. Moreover, these materials allow for the delivery of both hydrophilic and hydrophobic medicines [16, 17]. For the following reasons, injectable formulations of the different hydrogels that can be delivered in their sol state before gelation occurs have drawn a lot of interests [18-20]. To solve cell adhesion problems, it is easy and fast to use the combination of hydrogel precursor with cells and bioactive molecules [21-23]. Drug administration by injection and local dispersion reduces systemic toxicity [24, 25]. The physical or chemical crosslinking of polymers into gel could be established [26, 27]. Covalent linkages known as chemical crosslinks are generated as a consequence of chemical processes [28]. These gels are kept from being diluted by surrounding fluids, penetrating, or migrating out of the targeted location because chemical linkages are mechanically

stronger than physical ones. Furthermore, they might make physical touch with a broader range of tissues in a forceful and long-lasting manner [29]. Reversibility does not exist in these relationships, making them permanent. The majority of these gels prior use were for pre-formed implants; however, new procedures have made it feasible to inject them. Chemical processes do not contribute to the production of various types of crosslinks, which are physical and non-covalent [30]. The most important interactions that result in the development of gels in this class include hydrophobic interactions, dipolar-dipolar and hydrogen bonding [31-33]. Due to the reversible nature of these interactions, a substance can transition into a gel outside of the body, then break bonds upon injection into the body due to shear forces, resulting in a sol state, before finally returning to the gel state [34-36].

Cancer is one of the diseases accountings for a very high number of infections and deaths worldwide, such that one out of every five men and one woman is affected by it, and one out of every eight men and Every 11 women die [37]. This number is increasing annually due to worsening environmental factors. In the field of cancer treatment, injectable gels prevent systemic toxicity by being administered intratumorally. Cancer treatment can be done in different ways such as surgery, radiotherapy, chemotherapy, hormonal therapy, immunotherapy, hyperthermia therapy, photothermal therapy, gene therapy, photodynamic therapy and targeted therapy, among which chemotherapy is the most used. Chemotherapy is the use of drugs to destroy cancer cells with their toxic effect. Immunotherapy means strengthening the immune system to fight cancer. In this context, in spite of the local administration, it is possible to activate the systemic immune response. Different ways of this type of treatment include immune regulation, for example, with immune checkpoint drugs, cytokines or antibodies, delivery of immune cells and vaccines [38]. In general, polyphosphate, polyethylene glycol and polylactides glycolic acid are the most important polymers used in this field [37, 39]. In most of these cases, the ability of the system in cancer treatment can be greatly improved by functionalizing with target parts, delivering multiple drugs simultaneously, and using theragnostic systems [40].

Endogenous Stimuli

After injecting the gel and causing it to gel, the environment within the body applies internal stimulus [25, 41, 42]. In cancer treatment, the unique characteristics of cancerous tissues, such as their higher temperature or lower pH than healthy tissues, can be considered as stimuli for gelation [43, 44]. This category includes only physically based gels.

Temperature

In three different ways, temperature-responsive materials undergo the sol/gel phase transition. Several of these materials experience gelation when the temperature rises. The temperature of gelation is influenced by the number of monomers in the fluid. The lower critical solution temperature (LCST) is the temperature at which any chemical begins to gel at a specific concentration. As the temperature increases, these gels contract, which among other things, limits the release of any possible treatment, a property known as negative temperature dependence. As they dissolve and solidify once delivered to the body at roughly 37 °C, these gels are often utilized [45]. The hydrogen link between the groups of monomers and the water in the solution weakens as the temperature rises and along with the intensity of the hydrophobic contacts of the main chain produces gelation and contraction and the formation of an anhydrous polymer [46]. The most well-known member of this family, PNIPAM Poly (N-isopropyl acrylamide), has an LCST of roughly 32°C, and as it contracts, it switches from an extended helix form to a coiled-coil state. By copolymerizing this polymer with hydrophilic monomers, it is feasible to raise the gelation temperature, whereas hydrophobic monomers can lower the temperature. The usage of the first scenario is made possible by the gelation temperatures, which are both desired and near to body temperature. Its potential for being poisonous and non-degradable is one of the major drawbacks of this gel [47]. In contrast to the previous example, the second group of materials gels at a lower temperature. The need for a high-temperature solution prior to injection limits the usage of these materials since delicate medications and biomolecules may be harmed [48]. The third class specifically, when the temperature rises, a sol first turns into a gel and then, at higher temperatures,

a gel turns back into a sol. When the temperature climbs over the gel's LCST, the polymers in some amphiphilic members of this family of materials interact with one another to form monomers, which is then self-assembled. Once their fraction surpasses a particular threshold, they become micelles. The material turns into a hydrogel when the micelles are so closely packed and knotted that they are unable to move [49].

One of the most important formulations in this field is a triblock copolymer, which has the chemical formula PEO-PPO-PEO and whose most important component is poloxamer or Pluronic. Its side blocks are hydrophilic, and its center block is hydrophobic. Poloxamer 407 or Pluronic F127, on the other hand, is the least poisonous and most often used. Nevertheless, because the crosslinks between the micelles are weak, the material's mechanical qualities are poor. Moreover, the structure separates quickly and releases from them. In addition, these materials cannot degrade naturally, and these products may be made better by changing their structural design [50]. This approach includes drawbacks in addition to the advantages already discussed. Take a high gelation rate as an example. If that happens, the material inside the syringe needle may gel and prevent injection, or if the gelation happens slowly, the discharge of these compounds may temporarily reach a burst condition. In general, when it comes to block copolymers like poloxamer and polymers containing PANIPAAM, it is possible to work on the copolymer composition. In order to strengthen the mechanical stability and biocompatibility of Pluronic F127, Chen et al., added crosslinks using hexamethylene diisocyanate. In order to create a composite with a hyaluronic acid matrix, these nanostructures were employed. The viability of cancer cells and tumor growth were adversely affected by the anticancer medicine DOX when it was brought to the zero-order state using this chemical. Moreover, it was demonstrated that the concentration parameter of nanostructures may be used to control the rate of deterioration [51]. A PLGA-PEG-PLGA-based nano-scaled composite with DOX was developed by Tan et al., Great drug loading capacity, prolonged release, and both types of medicines' response to pH are benefits of this gel [52]. A thermos responsive injectable hydrogel based on chitosan was created by Ahsan et al., as a powerful anticancer medication carrier [53]. The findings show that for the long-term management of cancer, hydrogels containing disulfiram can be used as a strong injectable therapeutic agent (Figure 1).

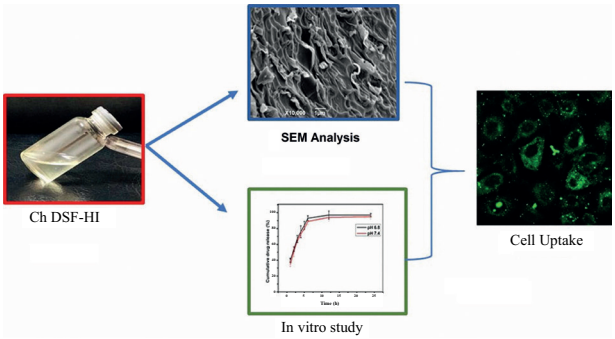

Figure 1: Schematic of Thermos Responsive Chitosan-Based Injectable Hydrogel [53] *(Source: Ahsan et al., 2020. License: This is an open access article published under an ACS AuthorChoice License, which permits copying and redistribution of the article or any adaptations for non-commercial purposes.)*

PH

PH stimulation is used for drug release and volume change in gels, while there are cases of gel induction. As chitosan is the only naturally occurring cationic polysaccharide and an N-deacetylated derivative of chitin, a pH shift is known to cause it to gel. The major amine groups left hanging after protonating this substance, a polybase, receive a positive charge and change their formula from -NH2 to -NH3+. To restore the initial state of the gel, hydrogen bonds are formed between the amine groups, but when the pKa point is reached, the positive charges are removed [54]. The acidic pH of the chemical in the sol form irritates the host tissue, which decreases as the gel breaks down. It is feasible to employ polymer blends and hydrophobic alteration to improve the overall strength of polymers. As tumors and inflamed areas have an acidic pH, these gels can be used in immunotherapy, gene therapy, and chemotherapy to treat cancer. For instance, anticancer drug-containing nanogels can be administered intravenously and can be engineered with target ligands to collect in the tumor region and form a bulk gel. The medicine is first released into a gel before reaching the target location, preventing systemic adverse effects. Ph-responsive groups can be added to gels from other categories, such as thermos responsive gels, to create dual-responsive

gels [55]. Li et al., created PEG derivatives and α, β-polyaspartic hydrazide during the injection. With rising pH, this hydrogel was capable of transforming into an acidic cell in cancer, releasing the medication DOX, and returning to its original gel condition. Testing of the final product on animals with human fibrosarcoma revealed that it was biocompatible and biodegradable (Figure 2)[56].

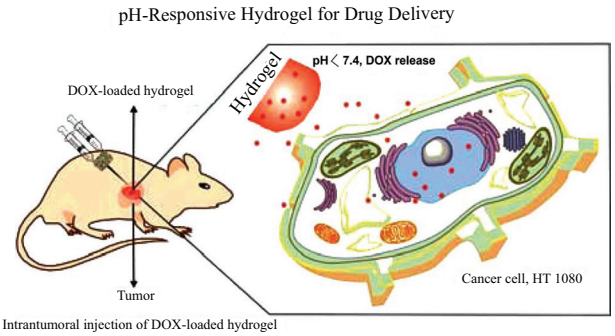

Figure 2: Schematic of an Injectable PH-Sensitive Hydrogel for Localized Fibrosarcoma Therapy. Adapted By Approval From [56] *(License: Reprinted (adapted) with permission from Li L, Gu J, Zhang J, Xie Z, Lu Y, Shen L, et al. Injectable and Biodegradable pH-Responsive Hydrogels for Localized and Sustained Treatment of Human Fibrosarcoma. ACS Appl Mater Interfaces. 2015;7(15):8033-40. DOI: 10.1021/acsami.5b00389 PMID: 25838258. Copyright 2015 American Chemical Society.)*

Enzyme

Transglutaminase enzymes, often referred to as bio adhesives, can help some protein hydrogels, including those made of gelatin, collagen, and fibrin, generate amide bonds between the protein molecules. This link has excellent stability in the body and is very resistant to proteolysis. One such enzyme, factor XIIIa, is responsible for the gelation of fibrin hydrogels. The horseradish peroxide (HRP) enzyme, which functions when an oxidant such H2O2 is present as a substrate, is another enzyme that is frequently utilized in the gelation of polysaccharide-based polymers. This enzyme-producing solid gels is stable and can be easily purified. Moreover, the reaction takes place at a mild temperature and neutral pH, and since the enzyme and substrate have very specified roles in the synthesis and there are no adverse side effects, no toxicity is produced. Oh et al., used a gelatin-based degradable gel with horseradish peroxidase and hydrogen peroxide to sustain deliver dendritic cells and oncolytic adenoviruses that express antitumor cytokines in an active form. As a result of the expression of these cytokines and the creation of tumor lysates, both indigenous and foreign DCs rapidly mature. Oncolytic adenovirus and this enhanced expression of cytokines further promote DC migration to draining lymph nodes, T cell activation, and tumor infiltration. Moreover, thymic atrophy and tumor-induced immune suppression are lessened by the production of Th1 cytokines [57].

Ion

Some polyelectrolytes, like alginate and pectin, dissolve in water and change into anions by, for example, losing the carboxyl group H. When these anions are joined by 2- or 3-valent counter-ions, such as Ca2+, the result is a gel. Injecting a polymer solution along with a CaCl2 solution or an aqueous slurry of Ca salts into the necessary area in this way involves the use of a two-syringe applicator. By changing the number of counter-ions, one may alter the crosslink density and the mechanical characteristics of the gel. On the other hand, a high concentration of the opposite ion damages cell survival and the therapeutic effect. As a result, this parameter's value is ideal. Salts of this ion with decreased solubility could be utilized to prevent the excessive raising of the Ca2+ concentration. Ca ions are released in water, for instance, by α-TCP. Using calcium-free gels is a different alternative. Pectin and alginate do not have strong cell adherence; however, this problem can be fixed with a few steps. Alginate gels also degrade slowly since no hydrolysis or enzymatic degradation occurs on these substances. Nevertheless, partial oxidation makes hydrolysis feasible and speeds up the pace of deterioration [58]. Charged tiny molecules have also been utilized for this kind of gelation in addition to ionic crosslinkers. Similar bonds are formed by these agents, however there are variances in penetration rates. There are, of course, other forms of ionic gelation, such as the formation of a polyelectrolyte complex by the interaction of two polyelectrolytes with opposing charges. The issue of quick gelation and syringe obstruction does not arise in these procedures because the mass transfer mechanism used for ion and pH stimuli gelation is slower than the heat transfer mechanism used for

temperature stimuli gelation.

Chao et al., used an alginate gel to deliver 131I radioisotope, immunostimulatory CpG oligonucleotide, and a checkpoint inhibitor drug. 131I was labeled on an enzyme that decomposes the endogenous hydrogen peroxide of the tumor into oxygen, and with a long-term oxygen supply, it causes more effective radioisotope treatment and complete removal of the tumor at low doses of radioactivity. CpG oligonucleotides activated the innate immune cell receptor and caused a strong proinflammatory response. Moreover, the use of an antibody to suppress CTLA-4 (cytotoxic T-lymphocyte-associated protein-4) stopped the spread of the tumor and its recurrence. Choosing the optimal concentration of alginate led to uniform distribution of radioisotopes in the tumor and no leakage to healthy tissues [59].

Exogenous Stimuli

The application of external stimuli causes the body to begin to gel [60-62]. The carrier may initiate gelation when it reaches the correct spot, and the process can be stopped after the necessary fraction has gelled, making them simple, quick, and accurate to regulate [63].

Photo

The gel network is created and destroyed by this stimulation. Initiation, propagation, and termination are the three phases of network development employing this method in the presence of visible or UV light. First, there are the drug's monomers or oligomers, as well as a substance known as a photo initiator, which employs regulated concentrations of photo initiators of the free radical kind without significantly affecting toxicity. In most cases, exposure to UV light results in the excitation and breakage of a particular bond in the photo initiator as well as the production of a free radical. Then, these free radicals break the double bonds in the primary chain of the monomers, resulting in the production of further free radicals. During the rapid propagation phase, they carry out the same process on the surrounding monomers in a chain reaction producing a long polymer chain with chemical connections. This gelation method is quicker than other methods; such gelation is brought on by temperature because of the UV light's high intensity, its rapid transmission of vast amounts of heat, and other variables. Regarding this, it is feasible to modify the light's intensity, the type and amount of photo initiators or to irradiate light sporadically while gelation is occurring. In order to control the rate of gelation, it is also possible to alter the number of reactive double bonds. The gel's crosslink density rises with a prolonged irradiation period. It works on the mechanical characteristics, swelling or deterioration, and biomolecule delivery of the gel. It restricts the movement of host and encapsulated cells as well as the flow of nutrients and oxygen. Surface polymerization can potentially be used to tackle this issue instead of bulk [64].

Due to the UV radiation and presence of free radicals, this technique has the potential to harm DNA, obstruct cell activity, and alter gene expression. Although using laparoscopic radiation instruments to apply this approach to locations far from the skin's surface is an option, because UV light has a limited penetration depth in biological tissue, it is typically employed for subcutaneous damages. To overcome these difficulties, high-intensity visible light, which penetrates deeper and produces less tissue damage, can be employed. Because of the poor UV penetration, generating a hard gel is very difficult. Fourniols et al., used a polyethylene glycol Di methacrylate gel to deliver the drug Temozolomide and treat Glioblastoma brain tumor. The gel was applied following surgery and tumor excision. Based on its chemical makeup and the size of its pores, it should both stop cancer cells from infiltrating the brain tissue and eradicate any cancer cells already existed, lowering the likelihood of a local recurrence. This gel and the UV light used to polymerize it are commercial and can be easily used [65]. Xia et al., incorporated meso-tetracids (1-methylpyridinium-4-yl) porphyrin (TMPyP) photosensitizer in an injectable gel composed of Di benzaldehyde-terminated telechelic poly (ethylene glycol) and glycol chitosan [66]. The findings demonstrated promising attributes that improved the anti-tumor capability and fluorescence emitting properties of the gel carrier, facilitating the amendment of imaging-guided photosensitive cancer treatment (Figure 3).

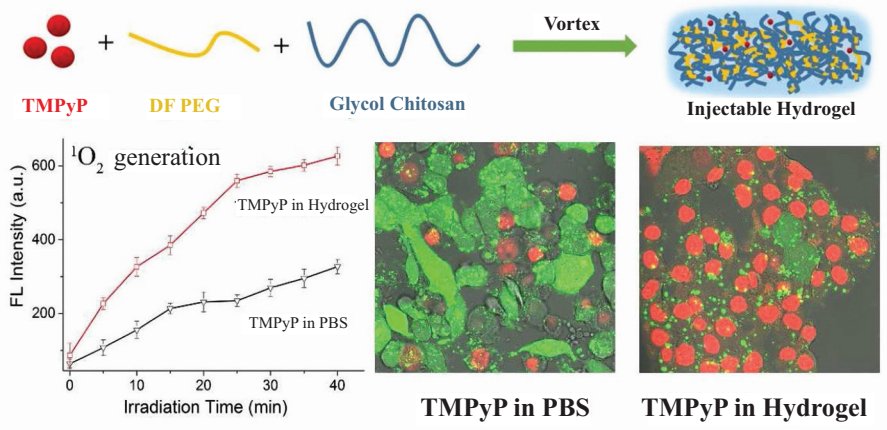

Figure 3: Illustration of Injectable Glycol Chitosan/Di Benzaldehyde-Terminated Telechelic Poly (Ethylene Glycol)-Encapsulated Meso-Tetrakis (1-Methylpyridinium-4-Yl) Porphyrin (Tmpyp) for Cancer Treatment. Adapted By Approval From [66] *(License: Reprinted (adapted) with permission from Xia LY, Zhang X, Cao M, Chen Z, Wu FG. Enhanced Fluorescence Emission and Singlet Oxygen Generation of Photosensitizers Embedded in Injectable Hydrogels for Imaging-Guided Photodynamic Cancer Therapy. Biomacromolecules. 2017;18(10):3073-81. DOI: 10.1021/acs.biomac.7b00725 PMID: 28820580. Copyright 2017 American Chemical Society.)*

Shear Stress

Shear-thin and self-healing compounds are the terms used to describe these substances. Shear force is used to reduce their viscosity during the injection. When the injection is finished, they turn into an injectable sol. Once the shear force is gone, they recover and return to being a gel. These substances are a subclass of self-healing substances in which the self-healing procedure is induced rather than taking place naturally. These substances possess form memory properties, meaning that after changing their shape, they will revert to their previous shape. If a fracture develops in one of the self-healing materials' components, it will be mended on its own, extending the lifespan of the material. These materials' components are connected by reversible physical linkages. The approach is unaffected by the problems with rapid and slow gelation because of its superior gelation speed. Such gels also lessen the impact of shear strain on cells, which is another benefit. Qu et al., developed a gel composed of PEG/chitosan composite for liver carcinoma therapy. When the pH of the gel was changed, the Schiff-base link between the two components was broken, causing the gel to disintegrate and the DOX medication to be released. L929 and HepG2 cells showed no toxicity toward the drug-free gel. Through varying crosslinker concentrations, gelation duration, storage modulus, and degradation may be controlled [67]. To treat cancer, infections, and wound healing, Wu et al., combined a unique copolymer with Schiff-base-bonded silica nanoparticles. The gel's mechanical qualities were easily enhanced by nanoparticles; they possessed a sufficient pKa, which made the gel stable at neutral pH and able to release the medication when exposed to slightly acidic conditions. Fast gelation, biocompatibility, and easy preparation were the advantages of this gel [68].

Magnetic Field

This type of gelation is performed by exploiting a varying magnetic field. In such a way that superparamagnetic iron oxide nanoparticles (SPIONs) are usually placed in the injection solutions, which quickly align with the application of the field, and if this field is alternating, it causes rotation and, as a result, the production of heat by these particles; This heat causes gelation in temperature-responsive gels. Exploiting SPIONs in glycerophosphate/chitosan gels, Zhang et al., suggested delivering Bacillus Calmette-Guérin, an immuno-treatment medication [69]. Gao et al., established a magnetic hypertonic saline-based implantable drug carrier for breast cancer thermos chemotherapy (Figure 4) [70].

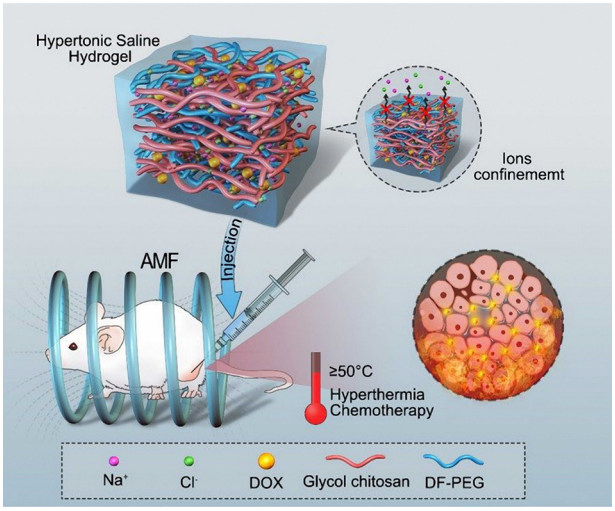

Figure 4: Schematic of Magnetic Hyperthermia-Mediated Breast Cancer Postoperative Recurrence Prevention. Adapted by Approval From [70] *(License: Reprinted (adapted) with permission from Gao F, Zhang T, Liu X, Ghosal A, Wang D, Xie W, et al. Nonmagnetic Hypertonic Saline-Based Implant for Breast Cancer Postsurgical Recurrence Prevention by Magnetic Field/pH-Driven Thermochemotherapy. ACS Appl Mater Interfaces. 2019;11(11):10597-607. DOI: 10.1021/acsami.9b02013 PMID: 30802401. Copyright 2019 American Chemical Society.)*

CONCLUSION

The liquid which can be infused through a body in a solution condition that afterwards is changed into a hydrogel as a consequence of an in-body stimulation is held in place by polymer networks in smart injectable gels. The introduction of medical drugs via a less invasive method is the main advantage of these materials. Several factors can turn these materials into gel, such as pH variations, changes in the concentration of polyvalent ions, UV radiation, decreases in shear stress and increases in gel viscosity or the creation of heat by nanoparticles in the temperature-responsive gel. This article intended to describe the merits, advantages, and disadvantages of each strategy. Despite the fact that these materials have several benefits in medical applications, some factors can help to improve their characteristics. While constructing gels, as with other biomaterials, it is crucial to take delicate chemicals like DNA, oligonucleotides, proteins, and peptides into account to make sure that the cells can continue to grow and function normally. Moreover, it is essential to look at whether catalysts or crosslinkers that have not yet reacted, degradation products, or residual components might cause cytotoxicity. It is crucial to consider how the drug interacts with the host tissue to reduce inflammation. The remodeling process, cell penetration due to cell signals, and proteolytic destruction of the gel are all motivated by biological events. Inside the gel, it is feasible to utilize proteins susceptible to metallo-proteinase. It should also be noted that when employing biodegradable materials for drug administration, the pace of degradation could be tuned by the release of the medication in question. On the other hand, we need to examine the target environmental factors, cellular processes, and metabolic activities in both healthy and pathological tissue states in more depth.

The next crucial factor in the formation of gels is rheology. The rate of gelation slows down if the material's viscosity is below a specific threshold, and high viscosity gels need more power to inject, which is uncomfortable for both the patient and the practitioner. Moreover, it is impractical to use this approach for deep target areas that demand lengthy material transit through the catheter. The transfer distance is shortened in this situation by utilizing a two-syringe instrument. These materials' rheology has to be precisely regulated in order to ensure homogenous material flow. One of the other difficult areas is mechanical characteristics, which may be strengthened in gels by using stronger gels, which likely have more compact networks. Nevertheless, in this instance, it is more difficult to penetrate the extruded flows' interface, and there is less chance of producing a homogenous network. On the other hand, the ideal gel needs to be flexible in order to stay intact once the invading cells are reorganized. We can list the short half-life of the hydrogels created thus far as one of their drawbacks. Moreover, numerous injections cannot be delivered to the intended area. Moreover, there is a chance that natural polymers might alter across batches, which would modify their origin-related features. Another difficulty is raising the GF concentration for human usage. As new materials with complex formulations are created, issues of safety and inconsistent physical quality arise. Hence, using straightforward formulas and straightforward production processes is always required. Nowadays, most of the in vivo research and experiments are done in subcutaneous ectopic tumor models to monitor the tumor condition more easily and administer the hydrogel inside or near

the tumor, but in this case, there is no specific microenvironment of the tumor itself. Investigating this situation in future works can be very helpful for the development and improvement of formulations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS APPROVAL

None declared.

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