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A REVIEW: A NOVEL GELATIN BASED DELIVERY SYSTEMS FOR GENE THERAPY AND PACLITAXEL TARGETING CANCER CELLS

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ABSTRACT

Gelatin is a natural biocompatible, nontoxic, edible and an inexpensive molecule. These properties result in its wide applications and recently it has been used for the delivery of the gene therapeutic entities such as plasmid DNA and paclitaxel drug based delivery for the various types of Cancer cells including Bladder cancer cells and prostate cancer cells. Generally, gelatin nanoparticles can be made via a simple coacervation method using water-miscible non-solvents for gelatin, like alcohols, or salts, like sodium sulfate. Since most of the effective non-viral gene carriers suffer from non-biodegradability and cell toxicity, these properties of gelatin have made it a promising choice for non-viral gene delivery systems. The preparation of paclitaxel-loaded gelatin nanoparticles and their biological activity in human bladder cancer cells and gelatin based delivery system for cancer gene therapy includes the non-viral vectors and the viral vectors which dominates in clinical trials. The paclitaxel-loaded gelatin nanoparticles represent a rapid release, biologically active paclitaxel formulation that can be used for intravesical bladder cancer. The hydrophilicity of the gelatin enhances fluid uptake, whereas the hydrophobicity of polymers such as poly (lactic acid) and poly (lactic-co-glycolic acid) limits diffusion which would result in the slower drug release. The future of these promising approaches lies in the development of better techniques for preparing drug-gelatin complex or gene-gelatin complexes with the added advantage of biosafety of the patients.

KEYWORDS

Cancer, Gelatin, Gene Therapy, Nanoparticles, Paclitaxel

INTRODUCTION

Cancer is a genetic multistage disease in which the cells undergo mutations of genes encoding the normal regulatory processes. The loss of regulatory control results in abnormal cell proliferation. As a result, replacing mutated genes particularly oncogenes and tumor suppressor genes, leading to the control of cell division, have been the major aim of cancer gene therapy [28]. The current scenario for bladder cancer consists of transurethral tumor resection of visible tumors, followed by intravesical chemotherapy to reduce disease recurrence or progression. Intravesical chemotherapy provides the advantage of selectively delivering drugs in high concentration to the tumor-bearing bladder while minimizing the systemic exposure.

Paclitaxel shows higher activity compared with other antimicrotubule compounds, such as vinblastine, against human bladder cancer cells[15]. A phase II study has shown that 24-hour intravenous infusion of paclitaxel produced 42% partial and complete response rate of advanced and/ or metastatic bladder cancer[22]. In Histoculture Study of human bladder tumors, a

2-hour treatment with paclitaxel was sufficient to cause inhibition of tumor cell proliferation and induction of apoptosis, with a greater apoptotic effect in the more rapidly proliferating tumors [3]. Paclitaxel is tightly bound to intracellular macromolecules such as tubulin and microtubules, thereby resulting in significant drug accumulation in tumor cells [23]. The intracellular drug accumulation and retention contribute to the antitumor activity, particularly the delayed activity, of paclitaxel[1, 2]. Finally, paclitaxel is known to cause apoptosis by p53-dependent and independent pathways and is therefore less dependent on the p53 status as compared with other agents such as Mitomycin C, which depends on a functional p53 pathway for apoptosis induction [6, 29]. Hence, paclitaxel presents a theoretical advantage in the treatment of bladder cancer that shows a high frequency of p53 [6, 27]. These characteristics make paclitaxel an attractive candidate for intravesical therapy.

Gelatin is chosen because of its biocompatibility gelatin is widely used as a stabilizer in vaccines and has been approved by FDA for extra vascular administration[2]. In addition, the hydrophilicity of gelatin is expected to facilitate the fluid penetration into the particles and thereby enhance the diffusion-mediated drug release at the target. The submicron particle size of gelatin nanoparticles further enhances the particle degradation rate and thereby enhances the drug release rate.

Gelatin is a perfect agent owing to the fact that it is natural, biodegradable, biocompatible in physiological environments, edible, water permeable, and insoluble in cold water, whereas completely soluble in hot water. Hence, gelatin is widely used in pharmaceutical, medical, cosmetic, and food products as gelling, thickening, binding, and stabilizing agent. Gelatin is a natural, biocompatible, and nontoxic macromolecule which is obtained by the partial hydrolysis of collagen derived from the skin, white connective tissue, and bones of animals [8, 12]. In fact, there are various types of gelatin based on the preparation methods from collagen, the source of collagen, conditions during extraction, pH, thermal history, electrolyte, and impurities or additives. On the whole, the major privileges of gelatin are its low toxicity[25, 26] which has been proven clinically, and its capability of preserving the bioactivity of the therapeutic agent to be delivered in vivo [10].

GELATIN

The word “gelatin” is derived from “gelatus” which means firm [11]. In fact, there are various types of gelatin based on the preparation method from collagen, the source of collagen, conditions during extraction, pH, thermal history, electrolyte, and impurities or additives. In general, gelatin obtained by an acid-treated hydrolysis is known as type A, and gelatin obtained by a base-treated process is known as type B. In addition, choosing the source of the gelatin (bovine, porcine) depends on the formulation and desired market of the end product [1].

Generally, gelatin nanoparticles can be made via a simple coacervation method using water-miscible non-solvents for gelatin[13], like alcohols, or salts, like sodium sulfate. This contributes to the separation of a liquid gelatin-rich phase which is in equilibrium with a liquid having less amounts of the polymer and eventually, the coacervate phase is hardened by physical or chemical compounds. Among the covalent cross-linkers, formaldehyde is the most important while other aldehyde includes furfural, acrolein, glutaraldehyde, and glyceryl aldehyde. A two-step desolvation method was another interesting technique for the preparation of gelatin nanoparticles [31]. In the presented process, low molecular weight fractions of gelatin were removed, resulting in

higher stability of the formulated particles compared with those obtained by a one-step desolvation method [5].

GELATIN BASED DELIVERY SYSTEMS FOR CANCER GENE THERAPY

The cationized gelatin hydrogels containing plasmid DNA enhances gene expression of the DNA subsequent to intramuscular implantation[7, 17]. The (polyethylene glycol)-modified gelatin nanoparticles were developed via pH- and temperature-controlled ethanol-water solvent displacement technique as long-circulating delivery system for hydrophilic macromolecules like DNA [13]. The two-step desolvation method to develop a gene delivery system based on cationized gelatin nanoparticles was studied which did not show any significant cytotoxic effects [31] and The effects of biodegradable cationized gelatin microspheres were investigated incorporating NK4 plasmid DNA on mice bearing Lewis lung carcinoma tumors. In general, NK4 acts against hepatocyte growth factor (HGF), which is known to have a critical responsibility in morphogenesis and regeneration of living systems. NK4 is also capable of suppression of the angiogenic effects of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) [17]. Namely, NK4 is a bifunctional molecule which not only acts as an HGF antagonist but also as an angiogenesis inhibitor. In practice, use of cationized gelatin microspheres was able to prolong the release of NK4 plasmid DNA, resulting in plasmid expression for a longer time, most probably as a consequence of plasmid DNA protection from DNase attack.

In addition, in vivo results revealed that both gene expression and gelatin microsphere degradation lasted nearly 28 days, suggesting that the expression of NK4 protein in blood circulation and cancer tissue depends on the degradation of the gelatin microsphere over the course of 28 days. Moreover, radio-tracing test of NK4 plasmid DNA incorporated in cationized gelatin microspheres following the subcutaneous injection into tissue around the tumor mass revealed that gene expression was located only at the injected site [18].

The gelatin-based nanoparticles are capable for systemic delivery of plasmid DNA encoding VEGF receptor-1 (VEGF-R1 or sFlt-1) [14] **Figure 2**. In general, VEGF over expressed by most types of cancers, results in angiogenesis, and is one of the most potent pro-angiogenic factors known. The plasmid DNA used encodes the extracellular domain of Flt-1 VEGF receptor excluding the transmembrane and cytoplasmic domains to entrap VEGF produced by tumor cells. They encapsulated the plasmid DNA with gelatin (Gel), thiolated gelatin (SHGel), and polyethylene glycol-modified gelatin (SHGel), and polyethylene glycol-modified gelatin (PEG-Gel), as well as with polyethylene glycol-modified thiolated gelatin (PEG-SHGel) as outlined in **Figure 1**.

Thus, these handful of studies, all performed in vivo, and showing efficacious potential of several anticancer genes delivered by a number of gelatin-based delivery systems summarized in Table, which highlight the latent promise in this class of vehicles for cancer gene therapy.

The gelatin based delivery system demonstrated that almost 13% - 15% of the recovered dose of and PEG-SHGel nanoparticles, respectively, accumulated in the tumor for up to 12 h following intravenous administration. On the whole, PEG-Gel nanoparticles may offer a safe and efficient strategy for systemic administration of therapeutic plasmid to solid tumors [14]. Furthermore, the cationized gelatin microspheres incorporating NK4 plasmid DNA, noticeably inhibited angiogenesis in Lewis lung

carcinoma tumor and also the suppression of disseminated pancreatic cancer cells in vivo studies. Finally, the results showed that subcutaneous injections of NK4 plasmid DNA immobilized within the cationized gelatin microspheres considerably suppressed the progression of disseminated pancreatic cancer cells in the peritoneal cavity of nude mice and Lewis lung carcinoma consequently prolonged animal survival [18].

Another application of gelatin in gene delivery utilized gelatin sponge particles (GSP) to increase transduction efficiency of adenoviral vector in catheter-mediated left hepatic arterial embolization (CHAE) in canine hepatocytes [20, 21]. The adenoviral vector expressing human HGF (Ad.hHGF) under the control of the cytomegalovirus promoter was used.

The cationized gelatin hydrogel were used as carrier for the delivery of plasmid DNA expressing small interference RNA (siRNA) for VEGF [19]. VEGF is one of the growth factor proteins known to possess an important role in vasculogenesis and angiogenesis. RNA interference (RNAi) is the sequence-specific gene-silencing method which is capable of binding and degrading target mRNA, similar to ribozymes and deoxyribozymes [4].

GELATIN BASED DELIVERY SYSTEMS FOR PACLITAXEL

The paclitaxel formulation approved by the Food and Drug Administration (FDA) for human use (e.g., Taxol) uses Cremophor to solubilize the drug. The paclitaxel is entrapped in the cremophor in micelles, reduces the free fraction of paclitaxel and consequently lowers the drug penetration into the bladder tissue [32]. Hence, the currently available paclitaxel formulation is not suitable for intravesical therapy. To overcome this problem firstly surface-active agent was used that is capable of disrupting the micelle structure and thereby increases the free fraction of paclitaxel; results show that dimethyl sulfoxide (DMSO) disrupts Cremophor micelles and restores the favorable bladder delviery of intravesical paclitaxel. However, because DMSO also increased the urine production rate and increased drug removal by the perfusing capillaries, the restoration by DMSO was incomplete [33]. Secondly the nanoparticles were prepared using several preparations of gelatin with different bloom numbers (75-100, 175, and 300) and using the desolvation method [30]. A higher bloom number corresponds to a higher molecular weight of the polymer. Gelatin nanoparticles were prepared by dissolving in water containing of 2% Tween 20, 20% aqueous solution of sodium sulfate followed by isopropanol containing 2 mg of paclitaxel [16]. A second aliquot of sodium sulfate solution was added until the solution turned turbid, which indicated the formation of gelatin aggregates. Adsorption of paclitaxel to nanoparticles was determined by incubating a trace amount of paclitaxel with empty gelatin nanoparticles [30]. Human RT4 cancer cells derived from a transitional cell papillary bladder tumor were cultured in McCoy's medium and were harvested from using trypsin and resuspended in fresh medium. Cells were seeded in 96-well microtiter plates (~2,000 cells per well) and allowed to attach to the plate surface for 24 hours. An aqueous solution of paclitaxel and paclitaxel-loaded gelatin nanoparticles were incubated with human bladder cancer RT4 cells [30]. Paclitaxel produces immediate and delayed cytotoxicity for immediate effective evaluation, cells were incubated with the culture medium containing aliquots of an aqueous solution of paclitaxel-loaded nanoparticles at equivalent paclitaxel doses for 48 and 96 hours, and the drug effect was measured immediately after treatment [9].

FUTURE DIRECTIONS

The preferred non-viral gene vector for cancer therapy should have low, if any, toxicity; be biocompatible and biodegradable; easily prepared and scaled-up at low cost; stable in storage; capable of particular gene expression in target cells; and have little, if any, interaction with plasma proteins. Gelatin, a biocompatible, natural, and nontoxic polymer, is one such candidate. Since present cancer gene therapy and drug therapy using gelatin is lacking in both efficiency and specificity in comparison with viral vectors, consequently, better approaches of preparing gelatin-gene complexes is required for better systemic gene therapy with this polymer. This will necessitate the improvement of further effective and patient-complaint techniques to enhance the potency of gelatin-based gene delivery systems compared to that of viral vectors.

Such development will be coordinated by improving the chemical structure of the polymer to make it more cationic, resulting in more efficacious interactions with anionic nucleic acid sequences and infected cancerous cells in the tissues. Another possibility is to engineer modified gelatin with the aim of targeting the gene to the cancerous cells and make certain that the desired plasmid DNA is just expressed at the target cell, for instance, by use of ligand complexes. The other approach could include the combination of gene therapeutic constructs with conventional cancer therapy like chemotherapy or radiotherapy. Finally, experiments in animals are required to evaluate the efficacy and safety of gelatin-based gene delivery systems in cancer therapy. Thus, in the not too distant future, we could probably witness several gelatin-based cancer gene therapy and drug therapy technology platforms clinically used.

SUMMARY

In view of the fact that most of the efficient non-viral gene carriers are non-biodegradable and toxic, gelatin is a promising choice due the fact that it is a natural, biocompatible, biodegradable and nontoxic polymer. Thus, gelatin not only fulfills wide-ranging functions as a gelling, thickening, binding, foaming, emulsifying, stabilizing agent matrix for implants and colloidal plasma expander, but also possibly be adapted into non-viral gene delivery agents. Numerous investigations have revealed the applicability of gelatin for gene delivery and drug delivery, and we have reviewed those that have used them in vivo and in vitro, and have related them to cancer gene therapy and paclitaxel- loaded gelatin nanoparticles represent a rapid release, biologically active paclitaxel formulation that can be used for intravesical bladder therapy.

Gene Type	Gene	Major indications	Cancer type	In vitro/ in vivo	Reference
Plasmid	NK4	- Suppression of angiogenesis, metastasis, and tumor volume - Increase of the cell apoptosis in the tumor tissue - Extension of the survival time	Lewis lung carcinoma	In vivo	Kushibiki et al. (2004a)
Plasmid	NK4	- Suppression of tumor progression and angiogenesis - Increase of the survival time - Extension of the survival time	Pancreatic cancer	In vivo	Kushibiki et al. (2004b)
Plasmid	VEGFR -1	- Dose-dependent tumor growth inhibition	Murine squamous cell carcinoma NRS-1	In vivo	Matsumoto et al. (2006)
Plasmid	sFlt-1	- Suppression of angiogenesis - Suppression of tumor growth	MDA-MB-435 human breast Adeno-carcinoma	In vitro/in vivo	Kommareddy & Amiji (2007
Plasmid	MMAC / PTEN	- Suppression of tumor growth - Increase of the cell apoptosis in the tumor tissue	PC3-Bcl-2 human prostate cancer cells	In vitro/ in vivo	Tomioka et al. (2008)
Adenoviral vector	G/Mphi/Adm 1L-12	- Suppression of tumor growth and spontaneous lung metastases	Prostate cancer cells	In vivo	Tabata et al. (2008)

Table 1:- Current gelatin-based drug delivery systems for cancer gene therapy (Somayeh Hallaj Nezhadi et al. 2009).

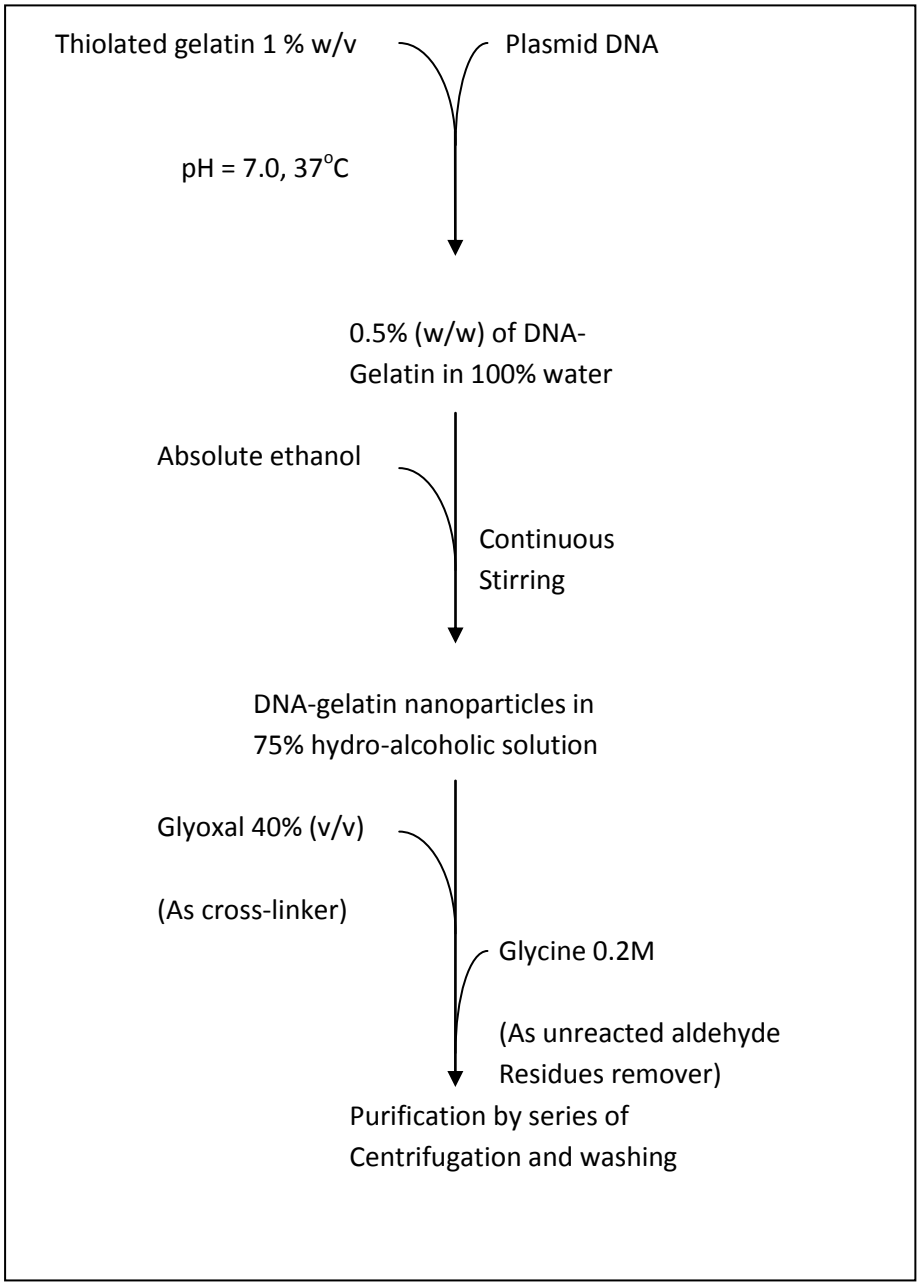

Figure 1: Diagram of the preparation of DNA-gelatin nano-spheres by controlled water-ethanol solvent displacement method (Kommareddy & amiji, 2007, Somayeh Hallaj Nezhadi et al. 2009).

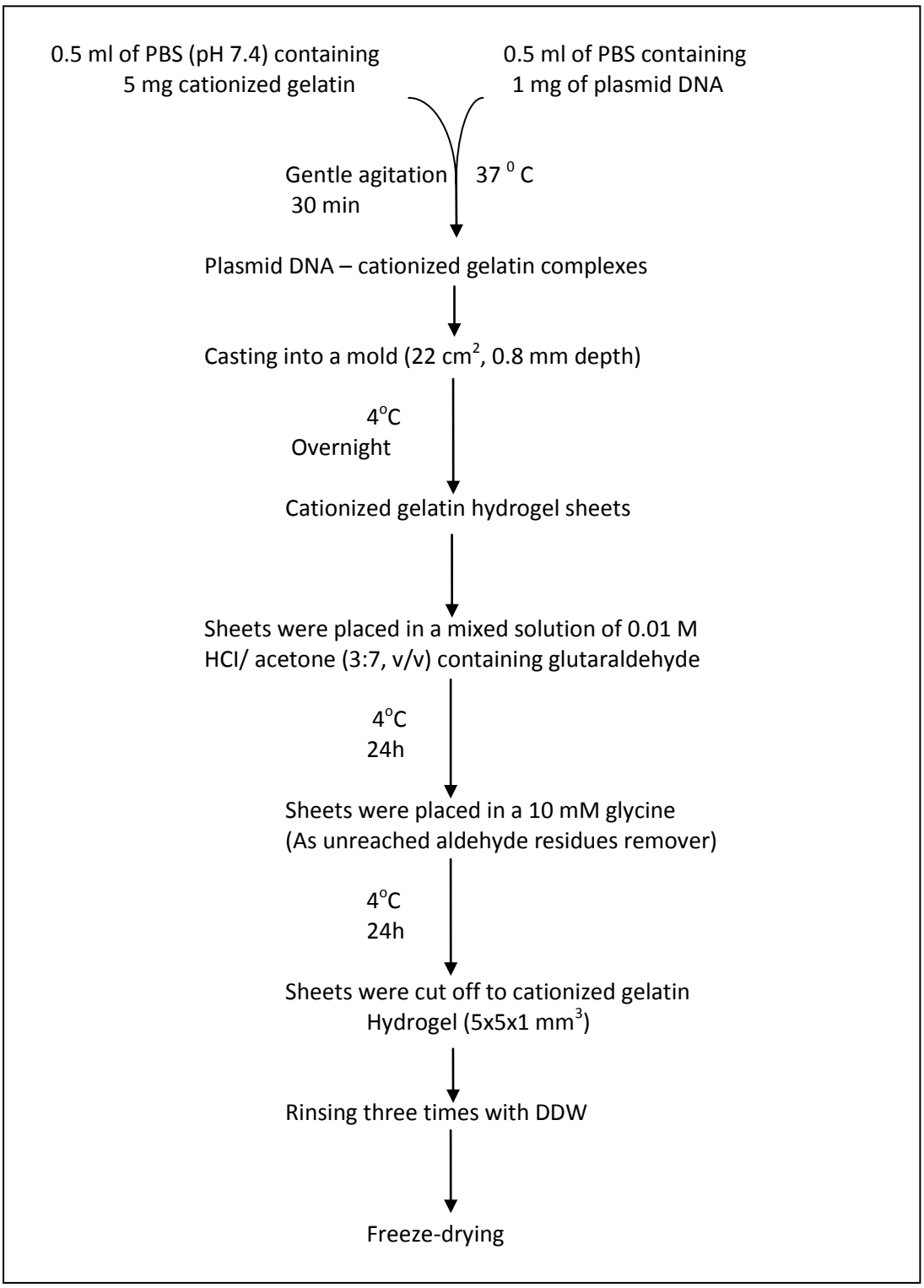

Figure 2: Diagram of preparation of cationized gelatin hydrogels (Fukunaka et al. 2002; Tomioka et al. 2008, Somayeh Hallaj Nezhadi et al. 2009).

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