# The Effectiveness of Urokinase and Vitamin C in the treatment of Arterial Occlusion Caused by Agarose Gel Injection

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**Abstract**

**Background and Aim: The agarose gel, which is a new generation of dermal filler, provides a great option for non-surgical aesthetic procedures. In this study it was aimed to determine the effectiveness of vitamin C and/or thrombolytic therapy in the complications that cause intraarterial occlusion of agarose gel.**

**Matarials and Methods: This study was carried out with a total of 36 adult male Wistar rats. Rats using defined flap model were divided into 4 groups as 3 experimental and 1 control. Agarose gel 1.5%, was injected into the left inferior epigastric artery from its bifurcation with the femoral artery and occlusion was achieved.A different drug was given to each group who underwent occlusion of the artery, and the drugs were injected intravenously through the tail vein 45 minutes after the artery blockage. Vitamin C and urokinase were injected into rats in Group A; Vitamin C was injected into Group B; Urokinase was injected into Group C and physiological serum was injected into Group D. Photographic measurements were made on the flaps and the necrotized and survived areas were statistically examined.**

**Results: It was found out that both the combination of Vitamin C and Urokinase and only Urokinase treatment significantly increased flap survival in the treatment of arterial agarose gel occlusion.**

**Conclusion: According to the results of the study it was concluded that the use of only urokinase was as effective as a combination of vitamin C and urokinase. The thrombolytic therapy alone could be effective for the treatment of arterial vascular occlusions caused by agarose gel.**

**KeyWords: Agarose gel, Urokinase, Vitamin C, Arterial Occlusion**

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## Introduction

Minimally invasive interventions applied for aesthetic purposes are practices that have increased in popularity in recent years. [1] According to The International Society of Aesthetic Plastic Surgery (ISAPS) International Survey on

Aesthetic Procedures[2] , more than 4,300,000 HA filler injections were performed by plastic surgeons worldwide in 2019 alone. This represents an 15.7% increase compared to 2018.

Dermal filler injections are amongst the most commonly performed procedures [1] As with all medical procedures, these applications have some potential side effects and/or complications. [3] These may depend on the procedure performed, the products or devices used, the technique applied, the personal characteristics of the patient, the anatomical region of the application and the other factors before and after the procedure.

Injection site reactions, hypersensitivity reactions, displacement of the filler, granuloma, biofilm, infection, papule and nodule formation are among the complications related to the filler. The most important complication is vascular complication. [4] Vascular complications can be local (Eg, tissue necrosis ) or they may develop in distant areas (eg: blindness, tissue necrosis) Local reactions can be caused by thrombosis, which develops as a result of injection of the filling material into the vascular structures, as well as compression, which develops as a result of an excessive amount of filler applied, can also lead to the appearance of these reactions.[5] Distant vascular complications are caused by the injection of filler material into the vein. [6]

In case of vascular complications, hyperbaric oxygen, anticoagulants such as aspirin, systemic antibiotic therapy, massage, vasodilator therapy and supportive treatments are needed.[7] If hyaluronic acid has been injected as filler, hyaluronidase can be used for the destruction of the filler material. [8]

Due to the complications that may occur as a result of cross-linked hyaluronic acid filler application, interest in researches about safer, fully biocompatible and biodegradable filler materials has increased.[9]

Agarose gel, a completely natural polysaccharide obtained by the high purification of red algae, is one of the fillers searched for this purpose. [10]It has been used clinically since 2006 and it is completely removed from the body by breaking down by macrophages, not by enzymatic means.[11] Its reliability has been proven by clinical studies. Agarose gel is removed from the body without leaving a residue, as it does not contain chemical cross-linking materials such as BDDE, PEG, which are found in crosslinked hyaluronic acid fillers. Since its chemical structure is hydrocolloid, not hydrophilic, the result of its volumizing effect is immediately visible. Due to its reticular structure, it has high Gprime and its migration is extremely low and lumps can be seen if it is injected in a bolus style.

Hyaluronidase, which is used to break down the hyaluronic acid filling in the polysaccharide structure, damages healthy tissues; leads to the destruction of tissues other than the filling and causes anaphylaxis. It is a product that cannot be used intravenously and is not licensed yet for filling demolition. [12,13]

Therefore, this study was designed to investigate the in vivo effects of thrombolytic Urokinase and vitamin C, which can be safely used in the lesion and intra arterially and is indicated to be able to wash thrombolytic and agarose gel, in complications that develop after excessive application to soft tissue or intra arterial injection. [14]

There is no safe intravenous agent that can wash the existing filler materials inside the vascular structures. The aim of this study is to investigate the effectiveness of vitamin C and/ or thrombolytic therapy as a destructive or therapeutic material that can be injected into the vein and used safely in the complications of intraarterial occlusion of agarose gel, which is a safe, promising new generation filling material.

## Materials and Methods

This study was carried out at Ege University Laboratory Animal Application and Research Center. In the study, 36 adult male Wistar rats weighing between 250-350 g were used. Rats were divided into four groups, three of which were designated as experimental groups (group A, B, and C) and one was designated as negative control group (group D).

Using the flap model defined in the rats, 50mg/kg Ketamine and 10mg/kg Xylazine were administered orally without any preoperative procedures; then, trichotomy was applied in both inguinal areas and femoral areas were after cleaning with betadine, a 2x2cm incision was made along the inguinal groove containing subcutaneous fatty tissue in a circumferential style, one centimeter of skin on the bottom lateral side of the flap was left connected to surrounding tissue without incision. This skin island was dissected retrogradely to the point of bifurcation of the superior epigastric artery/vein. In this way, an island flap based on a superficial epigastric artery/vein was obtained

(figure 1). After mixing 1.5% Agarose gel with 5 µm methylene blue for observation purposes, approximately 0.02 ml of agarose gel (Algeness® 1.5%, Advanced Aesthetic Technologies, Inc [AAT]) was injected into the left inferior epigastric artery with TSK 33G needle from its bifurcation with the femoral artery and occlusion was achieved (figure 1). On the right side, no action was taken for control observation.

A different drug was given to each group who underwent occlusion of the artery, and the drugs were injected intravenously through the tail vein 45 minutes after the artery blockage. Vitamin C and urokinase were injected into rats in Group A; Vitamin C was injected into Group B; Urokinase was injected into Group C and physiological serum was injected into Group D.

Following the procedure, paracetamol was given as 1mg/ml to drinking water on the first day in terms of postop analgesia. After flap adaptation in rats, flaps were monitored daily for necrosis development and Betadine dressing was applied. No movement restrictions were created for the rats. The rats were observed under the conditions of humidity and heat provided by the laboratory environment, in a 12-hour night-12-hour day cycle, under normal diet conditions and liquid that they can reach whenever they want.

In order to evaluate the formation of necrosis and demarcation in the flaps, the rats were observed for 7 days. Flaps were completely excised under anesthesia of 50 mg/kg Ketamine and 10 mg/kg Xylazine on the postoperative 7th.day and the vascular sections where agarose gel was injected were taken for histopathological examination. After that, the rats were sacrificed under high-dose anesthesia (100 mg/kg IP Ketamine). Photographic measurements were made on the flaps and the necrotized/and survived areas were statistically examined.

## Results

The mean flap survival rate among Group A (Vitamin C and urokinase) was 93.3%, Group B (Vitamin C) was 57%, Group C (Urokinase) was 92.3% and Group D (physiological serum) was 55.6%. (Table 1)

In this study, we determined that the combination of Vitamin C and Urokinase significantly increased flap survival in the treatment of arterial agarose gel occlusion. However only urokinase was as effective as the combination of vitamin c and urokinase. We also concluded that although the use of vitamin C alone was slightly effective compared to the control group, the difference was not significant (Table 1-Figure 2).

Due to the number of subjects used in the study, the Kruskal Wallis test, one of the non-parametric tests, was used in the analysis of the difference between the groups, without considering the normal distribution. For the same reason, Tamhane's was used in the post-hoc analysis. The results of the analyzes are summarized in Table 2. The difference between the groups was found to be statistically significant ($p<0.01$). As a result of Tamhane's used as post-hoc, there was a significant difference between Vitamin C + Urokinase group and Vitamin C and control groups ($p<0.01$), the difference between Vitamin C and Urokinase was significant ($p<0.01$). The difference between the control group was found to be insignificant ($p>0.05$). A significant difference was found between urokinase and the control group ($p<0.01$).

## Conclusion

Agarose gel is a new biologic, safe, long lasting, completely biodegradable, viscoelastic filler with excellent shaping and contouring capabilities.[7,13] Its special rheological characteristics and gel forming capability makes it a unique material. [14-16] and its hydrocolloid nature allows very precise injections with immediately visible result.[17] Although agarose gel is a completely natural and safe material for soft tissues, there is no previous study in the literature about the treatment options in the case of vascular occlusion.

In our experimental study we reached the conclusion that the use of only urokinase was as effective as a combination of vitamin c and urokinase, so thrombolytic therapy alone could be a very effective treatment option for the treatment of arterial vascular occlusions caused by agarose gel. Another interesting finding was that most of the flap area was survived in the untreated control group. This shows that the agarose gel is remarkably safe, even in cases of intravascular injection and this may be due to agarose gel's less inflamatuary, chemical free natural content.

Complications management is a critical part of clinical practice and physicians must have sufficient knowledge and skills in the identification and effective treatment of vascular occlusions. [18] For this reason, we think that whether isolated thrombolytic therapy can be a successful option for filling materials with different structures should be determined in future studies. Since the drugs used for thrombolytic therapy today are licensed, effective and safe agents, their successful use in the treatment of arterial agarose gel occlusion will be a very important development for the literature.

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## TABLES

Table 1. Flap survival ratio of the groups

| | Flap survival ratio % | | | |
|---|---|---|---|---|
| Rat No | Group A | Group B | Group C | Group D |
| | Vit C+ Urokinase | Vit C | Urokinase | Control |
| 1 | 86 | 48 | 92 | 62 |
| 2 | 92 | 57 | 89 | 46 |
| 3 | 95 | 59 | 76 | 55 |
| 4 | 100 | 62 | 98 | 52 |
| 5 | 91 | 41 | 96 | 60 |
| 6 | 85 | 60 | 100 | 48 |
| 7 | 100 | 68 | 94 | 61 |
| 8 | 95 | 57 | 92 | 59 |
| 9 | 96 | 61 | 94 | 58 |
| mean % | 93,3 | 57 | 92,3 | 55,6 |

**Table 2. Comparison of the groups**

| | Label | N | Mean Rank | Chi-Square | df | p |
|---|---|---|---|---|---|---|
| Group A[a] | Vit C+ Urokinase | 9 | 27,72 | 26,408 | 3 | 0,000 |
| Group B[b] | Vit C | 9 | 10,17 | | | |
| Group C[a] | Urokinase | 9 | 27,28 | | | |
| Group D[b] | Control | 9 | 8,83 | | | |

***The different letters on the group names show that there is a significant difference between the groups, while the same letters show that there is no significant difference)**

## FIGURES

Figure 1. Schematic diagram of the animal and flap model. Agarose gel injected into the left inferior epigastric artery from its bifurcation with the femoral artery (a) epigastric artery; (b) epigastric vein; (c) femoral artery; (d) femoral vein

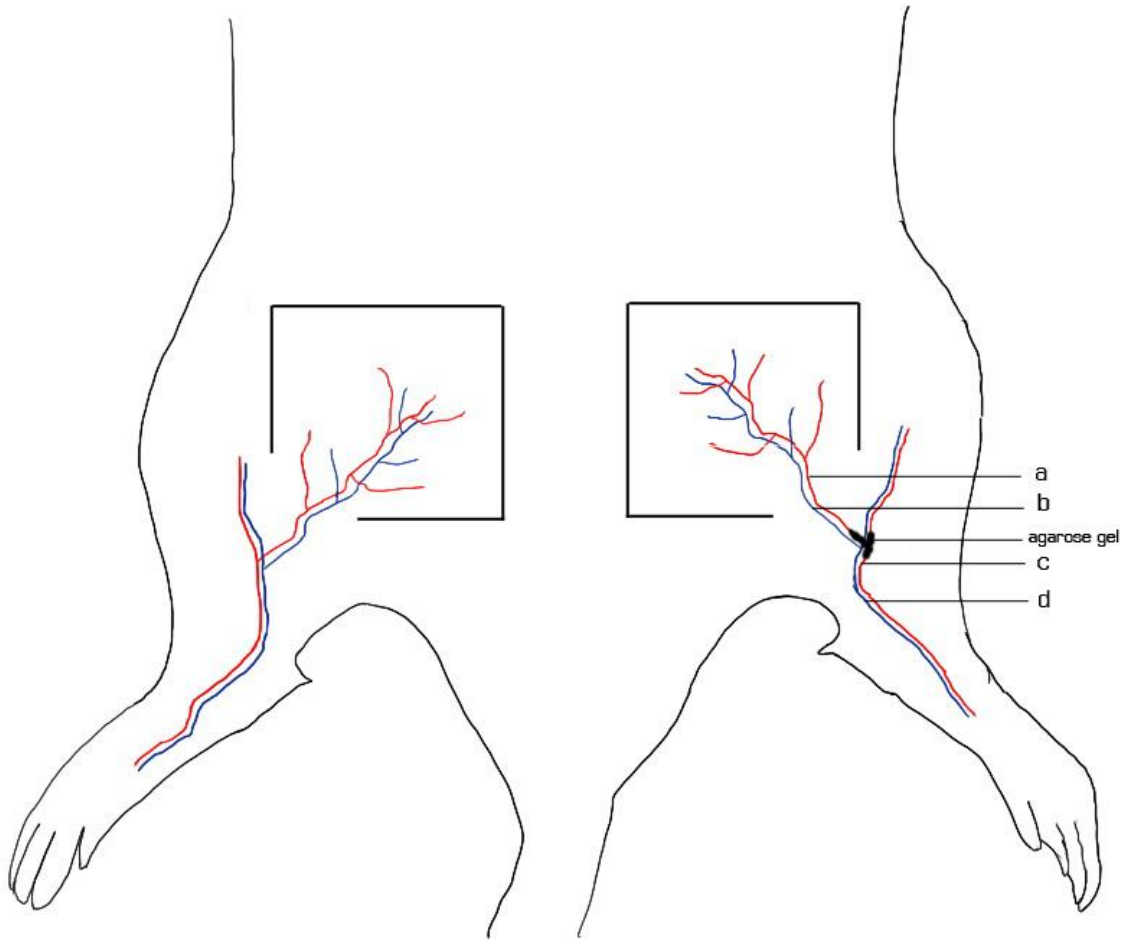


Figure 2. Flap necrosis in 4 experimental groups. (A) Vit C+ Urokinase; (B) Vitamin C; (C) Urokinase; (D) Control Group.