



Colposcopy and cytology after treatment with TIAB® system and hyaluronic acid-based vaginal capsules in patients who have undergone cervical surgery due to an HPV-related disease

Giada Lavitola¹, Nicoletta De Rosa¹, Ilaria Morra¹, Carmine Nappi¹, Giuseppe Bifulco¹

¹ Department of Obstetrics, Gynaecology and Urology, Federico II University Hospital Trust, Naples)

ABSTRACT

Introduction: LEEP (Loop Electrosurgical Excision Procedure) is the preferred technique for the treatment of high-grade precancerous cervical lesions (CIN2+). The post-operative follow-up provides for a colposcopy and cytology check-up 6 months after treatment. These examinations are designed to detect the presence of any residual disease. The surgical wound must have healed completely at the follow-up in order to obtain conclusive diagnostic results. This study aims to evaluate the efficacy of a topical TIAB® system and hyaluronic acid-based therapy in inducing complete tissue repair.

Materials and methods: 327 patients diagnosed with CIN2+ who had undergone LEEP were randomised into two groups. Group 1 (No. 152) received post-operative treatment with TIAB® system and hyaluronic acid-based vaginal capsules. Group 2 (No. 175) was the control group and did not receive any treatment. The patients underwent a colposcopy and cytology check-up at 1 and 4 months after treatment.

Results: at 1 month after treatment, the cytology assessment showed group 1 to have a higher percentage of satisfactory cytological specimens, a lower percentage of altered microbial flora and a higher incidence of complete maturation ($p < .001$). At the 4-month follow-up, it was found to have a lower percentage of altered microbial flora and a higher incidence of complete maturation ($p < .001$). The colposcopy carried out at 1 month shows a significantly higher percentage of inadequate results in group 2 than in group 1. At 4 months, 74.3% of colposcopies in group 2 showed the presence of immature metaplasia, which was significantly higher than in group 1 (3.3%; $p < .001$).

Conclusions: topical treatment with TIAB® system and hyaluronic acid-based vaginal capsules permits rapid restoration of the ectocervix following LEEP treatment. Complete tissue scarring allowed the satisfactory and adequate execution of the cytology and colposcopy examinations in 80.3% and 89.5% of cases respectively, at 1 month after follow-up, and a trophic squamous epithelium at 4 months after treatment.

Keywords: LEEP; colposcopy; cervical intraepithelial neoplasia; hyaluronic acid; TIAB® system.

SOMMARIO

Introduzione: la LEEP (Loop Eletrosurgical Excision Procedure) è la tecnica di elezione per il trattamento delle lesioni preneoplastiche del collo uterino di alto grado (CIN2+). Il follow up post-operatorio prevede un controllo colposcopico e citologico a distanza di 6 mesi dal trattamento. Tali indagini hanno come obiettivo di identificare la presenza di patologia residua. Una completa guarigione della ferita chirurgica al follow up è necessaria per ottenere esami diagnostici conclusivi. Obiettivo di questo studio è di valutare l'efficacia di una terapia topica a base di TIAB® system e acido ialuronico nell'induzione di una completa restituzione ad integrum dei tessuti.

Materiali e metodi: 327 pazienti con diagnosi di CIN2+ e sottoposte a LEEP sono state randomizzate in due gruppi. Il gruppo 1 (N= 152) ha eseguito nel post-operatorio una terapia con capsule vaginali a base di TIAB® system e acido ialuronico; il gruppo 2 (N=175) è stato considerato gruppo di controllo e non ha eseguito alcuna terapia. Le pazienti hanno quindi eseguito follow up citologico e colposcopico a 1 e a 4 mesi dal trattamento.

Risultati: a distanza di 1 mese dal trattamento il gruppo 1 ha dimostrato al referto citologico una percentuale maggiore di preparati citologici soddisfacenti, una più bassa percentuale di flora microbica alterata ed una più alta incidenza di maturazione completa ($p < .001$); al follow up eseguito al mese 4 viene dimostrata una più bassa percentuale di flora microbica alterata ed una più alta incidenza di maturazione completa ($p < .001$). La colposcopia eseguita al mese 1 dimostra una percentuale di referti inadeguati significativamente maggiore nel gruppo 2 che nel gruppo 1. Al mese 4, nel gruppo 2 la percentuale di colposcopie che rivelano la presenza di metaplasia immatura è del 74.3 %, significativamente maggiore rispetto al gruppo 1 (3.3%; $p < .001$).

Conclusioni: la terapia topica con capsule vaginali a base di TIAB® system e acido ialuronico consente una rapida restituzione ad integrum della portio a seguito di trattamento LEEP. La cicatrizzazione tissutale completa consente l'esecuzione di citologia e colposcopia soddisfacenti e adeguate nell'80.3% e nell'89.5% dei casi, rispettivamente, già ad 1 mese di follow up, ed un epitelio squamoso trofico a 4 mesi dal trattamento.

INTRODUCTION

Cervical intraepithelial neoplasia (CIN) are precursors to cervical cancer. As recommended by the US ASCCP⁽¹⁾ and Italian SICPCV⁽²⁾ guidelines, the preferred treatment for CIN2+ lesions is excision. LEEP is the most widespread technique used to treat these lesions.

Women who have undergone treatment for CIN face a 2-to-5-times higher risk of developing cervical cancer during their life time compared to the general population.⁽³⁾ Because of this, they must undergo the first cytology check-up with colposcopy at 6 months after treatment.⁽⁴⁻⁵⁾

Surgery aims to remove the abnormal tissue. Its extent depends on the extent of the lesion identified during the colposcopy and may vary between 7 and 25 mm in depth.⁽⁵⁾

The cervix has a high capacity for tissue regeneration. The data that demonstrates this derives from studies that have evaluated the post-partum tissue regeneration capacity of this region. It is the result of a series of biological processes that involve proinflammatory factors, metalloproteinases and proteins involved in the synthesis of the extracellular matrix.⁽⁶⁻⁸⁾

It has been shown that cervical tissue regenerates itself within 6 months of excisional surgery. Tissue regeneration is related to the quantity of tissue excised and exceeds 75% when the excised tissue accounts for less than 14% of the length of the cervix.⁽⁹⁾

Silver nanoparticles (AgNPs) have demonstrated bactericidal, virucidal and fungicidal activity. The patented TIAB® system contains titanium dioxide microcrystals with covalently linked silver ions.⁽¹⁰⁻¹¹⁾ The TIAB® system has also shown reepithelialising properties, inasmuch as it regulates collagen production.⁽¹²⁾

Hyaluronic acid (HA) is one of the main components in the extracellular matrix and plays an important role in regulating tissue repair. During the reepithelialisation process, high localised concentrations of HA stimulate the migration and proliferation of fibroblasts and the formation of granulation tissue, encouraging the deposit of collagen fibres and stimulating neoangiogenesis.⁽¹³⁾

This study aims to evaluate whether combined therapy with the TIAB® system and HA can accelerate the physiological tissue repair process in order to permit an early follow-up in patients who have undergone LEEP.

MATERIALS AND METHODS

From September 2013 to May 2015, all patients diagnosed with CIN2+ who needed excisional surgery at the Colposcopy and Cervical Pathology clinic were enrolled in a randomised prospective study. The inclusion criteria for enrolment were:

1. A positive histological diagnosis of CIN2+ following punch biopsy, in accordance with WHO criteria.⁽¹⁴⁾
2. Aged between 30 and 40 years old.
3. No medical history of systemic disorders such as: diabetes mellitus, thyroid disorder, blood clotting disorders.

Patients diagnosed with microinfiltrating or invasive carcinoma or pregnant patients with cervical disease were excluded from the study.

After having read and discussed the patient information sheet and agreed to participate in the study, all the eligible patients who met the inclusion criteria were enrolled and underwent a colposcopy and loop electrosurgical excision procedure (LEEP) with an RF diathermy loop.

The colposcopy was carried out using the standard technique: application of a vaginal physiological solution, application of 5% acetic acid, observation through the colposcope with a 10x and 40x magnifying lens, subsequent application of Lugol's iodine, followed by further observation. The colposcopic images were attached to the reports. The reports were written on the basis of the colposcopy criteria identified by the IFCPC in 2011.⁽¹⁵⁾

The surgery involved 4 phases:

1. Colposcopy
2. Radial infiltration with local anaesthetic (ropivacaine hydrochloride monohydrate 7.5) of the four quadrants of the ectocervix.
3. Tissue excision in one or two steps depending on the shape of the ectocervix and the extent of the lesion.
4. Vaporisation of the wound bed with ball electrode at a power of 70 Watts, with careful haemostasis control.

The surgical specimen went sent away on a support and fixed in formaldehyde 4% (DIAPATH). The sample was then sent to the Pathological Anatomy laboratory, where it was washed, included in paraffin and sectioned. Multiple 4 µm sections for each biopsy sample were stained with hematoxylin/eosin. The examined samples were classified in accordance with WHO criteria as negative, CIN1, CIN2, CIN3/Carcinoma in situ or microinvasive.⁽¹⁴⁾

The patients were then randomised into two groups: group 1 underwent treatment with TIAB® and hyaluronic acid-based vaginal capsules for 10 days a month for 4 months from the day after the operation. Group 2 did not receive any pharmacological treatment.

Patients in both groups then underwent a colposcopy and cytological sampling at 1 and 4 months after the operation.

The cytological sample was taken using the standard technique: application of a vaginal speculum, cleansing of the ectocervix with physiological solution, ectocervical sampling with an Ayre's spatula and endocervical sampling with a Cytobrush. The samples were rubbed on a slide, fixed with isopropyl alcohol - polyethylene glycols (GIMAFIX) and sent to the Pathological Anatomy laboratory. The samples then underwent standard staining according to the Papanicolaou method, before being examined.

For the purpose of evaluating ectocervical reepithelialisation, the cytology reports were categorised as follows:

1. satisfactory or unsatisfactory for reading (due to severe phlogosis, presence of red blood cells that obscured the specimen).

If the sample was inadequate for whatever reason (e.g. insufficient fixation, air drying artefacts or the presence of contaminants), the case was excluded from the study.

2. Presence or absence of altered lactobacillary microbial flora.
3. The degree of cellular maturation was then assessed:
 - a. Grade 1: reparatory metaplasia
 - b. Grade 2: complete maturation

The presence or absence of epithelial cellular anomalies was not considered as a classification criterion.

The colposcopy reports were also graded on the

basis of tissue maturation:

1. Inadequate colposcopy, incomplete scarring makes diagnosis impossible.
2. Colposcopic picture of acidophilia, suggestive of immature metaplasia.
3. Normal colposcopic picture of a completely mature trophic squamous epithelium.

Patients with abnormal changes were excluded from this study.

STATISTICAL ANALYSIS

SPSS 20.0 (SPSS Inc. Chicago, IL) software was used for the statistical analysis. The data distribution was evaluated using the Shapiro Wilks test. The Wilcoxon test and Student's t-test were used to compare the nonparametric and parametric continuous data between the groups. The Chi-squared test was used to compare the categorical data between the groups. The level of significance for all the tests was set at $p < 0.05$.

RESULTS

Three hundred and fifty-two patients met the inclusion criteria and were enrolled and randomised in the study. Of these, 327 patients completed the first and second colposcopy and cytology follow-up and were therefore included in the statistical analysis. 152 patients (group-1) received post-operative treatment with TIAB® system and hyaluronic-based vaginal capsules, while 175 patients did not receive any treatment (group 2, control). Twenty-five patients were excluded from the study: 14 patients were lost during follow-up, 4 patients did not take the prescribed treatment, 7 patients had positive colposcopy results at 1 month after treatment.

The patients' demographic data is summed up in **Table 1**.

Table 1
Demographic variables of the two study groups

	Group 1 (No.=152)	Group 2 (No.=175)	<i>p value</i>
Age (years)	33.7 ± 2.5	33.6 ± 2.4	NS
Parity (no.)	0.4 [0.3-0.5]	0.3 [0.2-0.4]	NS

Group 1: patients receiving post-operative therapy with the TIAB® system
Group 2: control group Mean ± sd; mean [CI, 95%]

Table 2 shows the cytology reports for the two study groups at 1 month and 4 months after surgery. A statistically significant difference was observed between the two study groups as regards the adequacy of the cytological specimen at the 1 month follow-up. The specimen was unsatisfactory in 40.6% of the untreated cases (group-2) and in 19.7% of the treated cases (group-1) ($p < .001$). A statistically significant difference was also demonstrated between the groups in the percentage of altered microbial flora and the maturation

index at both month 1 and month 4 (table 2), with a lower microbial alteration percentage and a higher complete maturation percentage in group 1. As regards the data concerning the colposcopy (table 2), a statistically significant difference was observed in the colposcopic picture between the groups at 1 month and 4 months from surgery. The percentage of inadequate colposcopic pictures at the first month of follow-up was 41.7% in the control group, while it was just 10.5% in the treated group (**Table 3**).

Table 2
Cytology reports for the two study groups at 1 month and 4 months after surgery

Cytology	Time 1			Time 1		
	Group 1 (No. = 152)	Group 2 (No. = 175)	<i>p</i> <i>value</i>	Group 1 (No. = 152)	Group 2 (No. = 175)	<i>p</i> <i>value</i>
Adequacy						
<i>Satisfaction</i>	122 (80.3)	104 (59.4)	<.001	144 (94.7)	159 (90.9)	<.001
<i>Unsatisfaction</i>	30 (19.7)	71 (40.6)		8 (5.3)	16 (9.1)	
Microbial flora						
<i>Normal</i>	141 (92.8)	128 (73.1)	<.001	145 (95.4)	145 (82.9)	<.001
<i>Altered</i>	11 (7.2)	47 (26.9)		7 (4.6)	30 (17.1)	
Maturation Index						
<i>Complete</i>	51 (33.6)	11 (6.3)	<.001	150 (98.7)	110 (62.9)	<.001
<i>Incomplete</i>	101 (66.4)	164 (93.7)		2 (1.3)	65 (37.1)	

Group 1: patients receiving post-operative therapy with the TIAB® system
Group 2: control group no (%)

Table 3
Colposcopy reports for the two study groups at 1 month and 4 months after surgery

Colposcopy	Time 1			Time 2		
	Group 1 (No. = 152)	Group 2 (No. = 175)	<i>p</i> <i>value</i>	Group 1 (No. = 152)	Group 2 (No. = 175)	<i>p</i> <i>value</i>
<i>Inadequate</i>	16 (10.5)	73 (41.7)	<.001	0 (0)	5 (2.9)	<.001
<i>Immature metaplasia</i>	121 (79.6)	99 (56.6)		5 (3.3)	130 (74.3)	
<i>Trophic original epithelium</i>	15 (9.9)	3 (1.7)		147 (96.7)	40 (22.9)	

Group 1: patients receiving post-operative therapy with the TIAB® system
Group 2: control group no (%)

DISCUSSION

Our findings demonstrate that combined topical treatment with TIAB® system and HA accelerates the cervical healing process in patients who have undergone LEEP due to CIN2+.

A significant increase was demonstrated in the percentage of cytology specimens adequate for examination after just one month of treatment and the percentage of adequate colposcopies was also significantly higher in the treated group. In fact, at 1 month from treatment, just 10.5% of cases in group 1 presented an inadequate colposcopy and only 19.7% of cases an inadequate cytological specimen. At 4 months, group 1 recorded 100% adequate colposcopies and just 5.3% cytological samples unsatisfactory for examination.

Spontaneous bleeding, partial cervical deepithelialisation and excessive inflammatory infiltrate are the conditions that have the most effect on misdiagnosis during colposcopy and cytology.

Treatment with TIAB® system and HA also reduces the number of cases with altered microbial flora at both 1 month and 4 months from surgery and this probably contributes to tissue maturation, with a positive effect on the cytological specimen.

The cervical squamous epithelium is the first and most important form of innate immune response in the genital environment, acting as a mechanical barrier that protects against attacks by opportunist pathogens.⁽¹⁶⁾ In the absence of this barrier and in the presence of favourable factors such as cervical bleeding as a result of surgery, which alters the vaginal pH, various pathogens can reproduce more easily and alter the normal resident microbiota. The onset of infection in a region undergoing active cervical reepithelialisation can delay the scarring process, interfering with normal cellular maturation processes.

The antimicrobial and antibacterial power of the TIAB® system would probably tend to restore balance to the local microbial flora, preventing contamination with other microbial forms.

The diagnostic and therapeutic procedure for CIN lesions does not involve cervical swabs being taken. The presence of infection is therefore not a decisive factor when choosing the most opportune time for treatment. Post-operative therapy with a drug that has antibacterial and antimycotic properties can reduce the risk of developing infections during the post-operative period and definitively delaying prompt and proper healing.

Wound healing is a complex process, characterised by angiogenesis, granulation tissue formation, collagen depositing, epithelialisation and wound contraction.⁽¹⁷⁾ AgNPs have been shown to promote cutaneous wound healing by means of anti-inflammatory activity.⁽¹⁸⁾ What is more, it has been demonstrated in animals and in the presence of an excisional wound, that AgNPs contribute positively to the reepithelialisation process, boosting fibroblast activation.⁽¹⁹⁾

Hyaluronic acid also possesses specific reepithelialising properties, leading to fibroblast proliferation and constituting a hydrophilic network at the level of the extracellular matrix that facilitates the transport of nutrients and metabolites that encourage faster tissue regeneration.⁽¹³⁾

Our findings show that topical therapy with the TIAB® system and HA encourages faster reepithelialisation and squamous cervical tissue maturation. At the 4-month follow-up, the group of treated patients showed a trophic squamous epithelium in 97% of cases, unlike the untreated subjects, who presented immature metaplastic tissue in 74% of cases and completely mature tissue in just 23% of cases.

In terms of clinical impact, this data suggests that in patients who require an earlier follow-up so as to rule out the persistence of the disease, treatment with the TIAB® system and HA could provide certain benefits with a lower risk of uncertain or inadequate cytology and colposcopy results.

It could therefore be of particular benefit to immunodeficient patients or those receiving therapy with immunosuppressants, so that they can resume therapy that is generally suspended in the presence of HPV-related disease as soon as possible, and to patients trying to conceive, so that they do not have to delay for excessive periods of time. In fact, while CIN is a condition that can develop at any age, in the natural history of the infection and carcinogenesis the peak incidence of CIN2-3 occurs in women aged between 25 and 35, a period that coincides with the childbearing age in western countries.⁽²⁰⁾

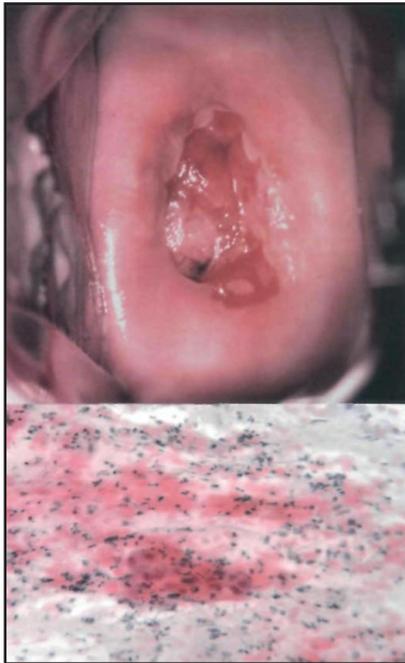


Fig. 1
Colposcopic picture (a) and cytological picture (b) at 1 month from excisional surgery in a control group patient (group 2).
a) A large area of deepithelialisation can be observed, with spontaneous bleeding.
b) The red blood cells obscure the view. Specimen inadequate for examination.



Fig. 2
Colposcopic picture (a) and cytological picture (b) at 4 month from excisional surgery in a control group patient (group 2).
a) Treatment outcomes can be observed with a periorificial metaplastic epithelium.
b) Reactive cellular changes caused by phlogosis. Enlarged nuclei and perinuclear halos with cytoplasmic vacuolations can be observed.

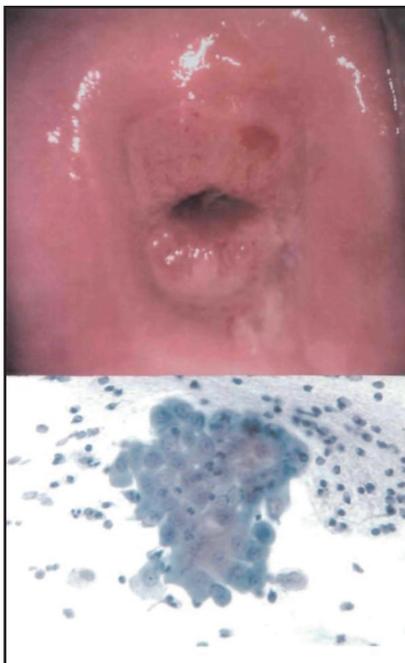


Fig. 3
Colposcopic picture (a) and cytological picture (b) at 1 month from excisional surgery in a group 1 patient
a) Cervical reepithelialisation can be observed with metaplastic squamous epithelium.
b) Squamous epithelium with typical repair, associated with mild phlogosis. Enlarged nuclei can be observed with evident nucleolus. Basophilic cytoplasm.

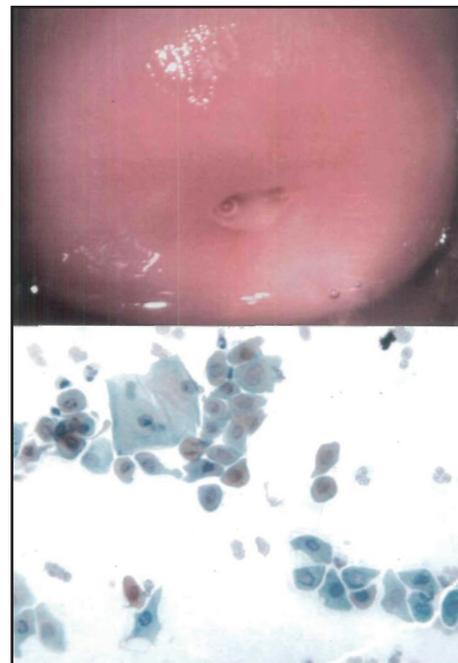


Fig. 4
Colposcopic picture (a) and cytological picture (b) at 4 months from excisional surgery in a group 1 patient.
a) Complete reepithelialisation can be observed with a trophic and mature squamous epithelium.
b) Mature squamous metaplasia

REFERENCES

- 1) Massad LS, Einstein MH, Huh WK, et al. **2012 ASCCP Consensus Guidelines Conference 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursor.** J Low Genit Tract Dis. 2013 Apr;17(5 Suppl 1):S1-S27.
- 2) **Gestione della paziente con Pap Test anormale.** Linee Guida Edizione 2006. Società italiana di Colposcopia e Patologia Cervico Vaginale.
- 3) Strander B, Andersson-Ellström A, Milsom I et al. **Long term risk of invasive cancer after treatment for cervical intraepithelial neoplasia grade 3: population based cohort study cervical intraepithelial neoplasia grade 3.** BMJ, 2007, 335(7629): 1077.
- 4) Arbyn M. Et al. **"EDS. European Guidelines for quality assurance cervical cancer screening."** Cap.6: management of abnormal cervical-cytology. 2Ed. Bruxelles. European Community.
- 5) **NHS Cervical Screening Programme. Colposcopy and Programme Management.** NHSCSP Publication number 20 Third Edition March 2016.
- 6) Timmons BC, Mahendroo M. **Process regulating cervical ripening differ from cervical dilation and postpartum repair: insights from gene expression studied.** Reprod Sci. 2007; 14:53-62.
- 7) Mahendroo M. **Cervical remodelling in term and preterm birth: insights from an animal model.** Reproduction. 2012; 143:429-438.
- 8) Stanley R, Ohashi T, Mowa C. **Postpartum cervical repair in mice: a morphological characterization and potential role for angiogenic factors.** Cell Tissue Re. 2015 Oct;362(1):253-63.
- 9) Papoutsis D, Rodolakis A, Mesogitis S, Sotiropoulou M, Antsaklis A. **Regeneration of uterine cervix at 6 months after large loop excision of the transformation zone for cervical intraepithelial neoplasia.** BJOG. 2012 May;119(6):678-84.
- 10) Lara HH, Romero-Urbina DG, Pierce C, Lopez-Ribot JL, Arellano-Jiménez MJ, Jose-Yacamán M. **Effect of silver nanoparticles on candida albicans biofilms: an ultrastructural study.** J Nanobiotechnology. 2015 Dec 15;13-91.
- 11) Orłowski P, Tomaszewska E, Gniadek M, Baska P, Nowakowska J, Sokolowska J, Nowak Z, Donten M, Celichowski G, Grobelny J, Krzyzowska M. **Tannic acid modified silver nanoparticles show antiviral activity in herpes simplex virus type 2 infection.** PLoS One. 2014 Aug 12;9(8):e104113.
- 12) Kwan KHL, Liu X, To MK, Yeung KW, Ho CM, Wong KK. **Modulation of collagen alignment by silver nanoparticles results in better mechanical properties in wound healing.** Nanomedicine. 2011 Aug;7(4):497-504.
- 13) Liang J, Jang D, Noble PW. **Hyaluronan as a therapeutic target in human disease.** Adv Drug Deliv Rev. 2016 Feb 1;97:186-203. Doi:10.1016/j.addr.2015.10.017. Epub 2015 Nov 2.
- 14) Wells M. **Epithelial tumors.** In: Tavassoli FA, Devilee P. editors. **World Health Organization classification of tumors: pathology and genetics of tumors of the breast and female genital organs.** Lyons, France: IARC Press; 2003. P. 269-70.
- 15) **2011 Colposcopic Terminology of the International Federation for Cervical Pathology and Colposcopy.** Bornstein J et al. Obstet Gynecol 2012. Jul;120(81):166-72.
- 16) Hickey DK, Patel MV, Fahey JV and Wira CR. **Innate and adaptive immunity at Mucosal Surfaces of the Female Reproductive Tract: Stratification and Integration of Immune Protection against the Transmission of Sexually Transmitted Infections.** J Reprod Immunol. 2011 March;88(2):185-94.
- 17) Midwood KS, Williams JE, Schwarzbauer JE. **Tissue repair and the dynamics of the extracellular matrix.** Int J Biochem Cell Biol, 36 (2004), pp. 1031-1037.
- 18) Tian J, Wong KK, Ho CM, Lok CN, Yu WY, Che CM et al. **Topical delivery of silver nanoparticles promotes wound healing.** ChemMedChem, 2 (2007), pp. 129-136.
- 19) Liu X, Lee PY, Ho CM, Lui VC, Chen Y, Che CM et al. **Silver nanoparticles mediate differential responses in keratinocytes and fibroblasts during skin wound healing.** ChemMedChem, 5(2010), pp. 468-475.
- 20) Kyrgiou M, Koliopoulos G, Martin-Hirsch P, Arbyn M, Pren-diville W, Paraskevaidis E. **Obstetric outcomes after conservative treatment for intraepithelial or early invasive cervical lesions: systematic review and meta-analysis.** Lancet 2006;367: 489-98.