

SUMMARY AND CONCLUSIONS

I. Summary

Diabetes is a multisystem disorder and complications of diabetes induce physiological changes in tissues and cells that impair the normal healing process. The pathophysiologic relationship between diabetes and impaired healing is complex. The diabetic wounds get stuck in the inflammatory phase featured by continuing influx of neutrophils that release cytotoxic enzymes.

Wounds that are difficult to heal represent a serious public health problem. The lesions severely affect the quality of life of individuals due to decreased mobility and substantial loss of productivity; they can also cause emotional damage and contribute to increase the burden of public expenditures in healthcare.

Physical treatments such as therapeutic ultrasound, Laser and electrotherapy are cited likewise in the literature as important adjuncts in wound management. These therapies seem to be advantageous but they have limitations and do not always achieve satisfactory results.

In recent years nanotechnology has been emerging as a rapidly growing field with numerous applications in science and technology.

This work aimed to evaluate the role of antibacterial nanofibers by incorporating silver nanoparticles into nanofibers using electrospinning as wound dressing on excisional wound healing dynamics in diabetic mice in comparison with physical methods such as therapeutic ultrasound and photostimulation using laser.

In the present study, we prepared different types of nanomaterials such as Ag nanoparticles using chemical reduction process, CA nanofibers and Ag loaded CA nanofibers using electrospinning process. The prepared Ag nanoparticles were characterized using Transmission Electron Microscope (TEM) to determine the morphology and the size of nanoparticles, X-ray Diffraction (XRD) pattern in order to evaluate crystalline state and Particle Size Analyzer (PSA) determine the particle size distribution curve of Ag nanoparticles.

The prepared CA and Ag loaded CA nanofibers were characterized in terms of morphology by scanning and transmission electron microscope, Fourier-transform Infrared spectroscopy (FTIR) to investigate functional groups. The antibacterial activity Ag loaded CA nanofibers with (1, 3 and 5 %) AgNO₃ using Disc Diffusion test and Growth kinetics study was determined.

Accordingly Ag nanoparticles, CA and CA loaded with 5% used for wound dressing in excisional wound of diabetic mice in comparison with physical therapy as follow:

- Group (I):** Diabetic free served as control group (10 mice) injected with citrate buffer.
- Group (II):** Experimental group (60 mice) injected with Streptozotocin, this group will be divided into the following subgroups:

IIa- Experimental group (10 mice), their open wounds left without treatment.

IIb- Experimental group (10 mice), their open wounds treated with Ag NPs only (50 μ L every other day).

IIc- Experimental group (10 mice), their open wounds dressed with CA nanofiber only.

IId- Experimental group (10 mice), their open wounds dressed with CA nanofiber contain AgNPs.

IIe- Experimental group (10 mice) treated with insulin combined with laser (650 nm, 150 mW) with a fluence of 5 J/cm² 5 minutes daily, for 10 days.

II f- Experimental group (10 mice) treated with insulin combined with pulsed ultrasound (0.5 W/cm²) 5 minutes daily, for 10 days.

- The healing was noticed and assessed during the treatment period and the Healing Factor Was determined.
- Histopathological Examination of mice skin during period of healing was done by taking skin from each group in 4th, 10th, 15th days of treatment period.
- Taking after healing period and determine the mechanical properties of healed skin

The results of the present work are:

For Ag nanoparticles they were spherical in shape and there is a variation in particle sizes ranging from 4.32nm to 7.27 nm with an average size 5.79 nm, from the XRD pattern it is clear that silver nanoparticles were essentially crystalline in nature and from particle size analyzer they were in a range of 29-41 nm. The highest fraction of Ag NP present in the solution was of 33.3 nm.

Good quality nanofibers for CA and Ag loaded CA nanofibers with smooth surface were obtained for CA and rough surface for Ag loaded CA nanofibers due to presence of Ag nanoparticles. FTIR showed that there are no major difference between the two samples in the major peak bands. However, it is also seen that the intensities of some peaks increase, this can be attributed to the presence of Ag NPs. The antibacterial activity of CA loaded with Ag Nanoparticles indicated high activity of CA loaded with 5% Ag nanoparticles.

Wound dressing using nanofibers loaded with Ag NPs improve the skin wound healing and the mechanical properties of the treated skin of the diabetic mice. Marked improvement in wound strength and healing due to increase in formation of collagen fibers and activity of the epithelial covering in mice receiving STZ and treated with insulin in combination with dressing with nanofibers loaded with Ag NPs.

Healing factor of different group increases with increasing of the treatment time. Healing factor of the diabetic mice treated with insulin dressed with nanofibers loaded with Ag NPs shows a healing factor higher than that of the normal group.

By comparing results obtained from different treatment modalities, we found that all therapeutic tools facilitate and accelerate diabetic wound healing, and improving scar quality but with different degree, and the best method is to use nanomaterials (nanofibers in conjugation with antimicrobial nanoparticles) which act as scaffold.

Conclusion

- 1- Nanotechnology is a very promising field that can be used in different application and science like in medicine.
- 2- Nanomaterials especially metal nanoparticles and nanofibers are wonderful materials that have unique properties than their original materials.
- 3- Abnormal wound healing that can occur during many pathological states like in diabetic patients is a very dangerous and can lead to mortality in some cases, can be treated using new therapies like using of nanotechnology.

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المخلص العربي

يعتبر مرض السكري من الأمراض الخطيرة المنتشرة بكثرة وتكمن خطورتها لما لها من مضاعفات وخاصة إذا تعرض مريض السكري لجروح فإن التئام هذه الجروح من المشاكل الصعبة التي يواجهها المريض ويسبب ذلك حدوث قرح في الجاد يصعب علاجها. ارتفاع تكاليف رعاية جروح مرضى السكري يزيد من الاعباء التي يتكبدها المرضى والمجتمع لذلك فإن هناك حاجة لتطوير طرق علاج جديدة، وغير مكلفة وأمنة تزيد من التئام جروح مرضى السكري وتعتبر تكنولوجيا النانو من أهم التقنيات في القرن ٢١ لما لها من تطبيقات واسعة النطاق في جميع المجالات.

ولقد تمكن العلماء من تطبيق هذه التكنولوجيا في المجال الطبي وذلك بتصنيع مواد لها أبعاد نانومترية و صفات فريدة تختلف في خواصها عن المواد ذاتها بأبعادها العادية. ومن هذه المواد هناك مواد يطلق عليها الألياف النانومترية التي يمكن استخدامها كضاد للجروح وكذلك يمكن دمج بعض المواد فيها مثل جزيئات الفضة النانومترية لإستخدامها كمضاد للبكتريا التي يمكن ان تلوث الجروح.

الهدف من الرسالة:

كان الهدف من هذه الرسالة هو تقييم دور الغشاء النانومتري المصنع من اسيتات السليلوز والمحتوي علي جزيئات الفضة النانومترية المضادة للبكتريا المصنع باستخدام طريقة الغزل الكهربائي في تصنيع والتئام الجروح في الفئران المصابة بالسكري مقارنة بالطرق الفيزيائية مثل الموجات فوق صوتية والليزر.

لتحقيق هذا الهدف تم

- ١- تحضير مواد نانومترية مثل جزيئات الفضة النانومترية المتكونة عن طريق الإختزال الكيميائي لجزيئات نترات الفضة، والغشاء النانومتري المصنع من اسيتات السليلوز والمحتوي علي جزيئات الفضة النانومترية عن طريق تحضيرها باستخدام طريقة الغزل الكهربائي لإستخدامهم كضاد في التئام الجروح ودراسة خواص هذه المواد كالآتي:
 - دراسة السطح الخارجي وحجم جزيئات الفضة النانومترية باستخدام الميكروسكوب الإلكتروني النافذ.
 - دراسة التركيب البلوري لجزيئات الفضة النانومترية باستخدام جهاز حيود الأشعة السينية.
 - دراسة السطح الخارجي للغشاء النانومتري و الغشاء النانومتري المصنع من اسيتات السليلوز والمحتوي علي جزيئات الفضة النانومترية المضادة للبكتريا عن استخدام الميكروسكوب الإلكتروني الماسح.
 - دراسة المجموعات الفعالة للغشاء النانومتري و الغشاء النانومتري المصنع من اسيتات السليلوز والمحتوي علي جزيئات الفضة النانومترية المضادة للبكتريا باستخدام جهاز الأشعة تحت الحمراء.
 - دراسة تأثير الغشاء النانومتري المصنع من اسيتات السليلوز والمحتوي علي جزيئات الفضة النانومترية المضادة للبكتريا في تثبيط نمو البكتريا ونشاطها.
 - والفئران المستخمة و عندها ٧٠ فأر تم حقن ٦٠ فأر منهم بمادة (straptozotocin) بجرعة 180 mg/kg حتي يتم اصابتهم بالسكري و ١٠ المتبقين لم يتم حقنهم ليكونوا المجموعة الضابطة ثم جرح جميع الفئران و عمل جرح دائري في منطقة الظهر واستخدمت الطرق المختلفة للمساعدة في التئام الجرح و تقسيم الفئران الى مجموعات كالآتي:
 - المجموعة الاولى : المجموعة الضابطة. فئران خالية من السكري تركوا بدون علاج لملاحظة التئام الجروح طبيعيا.
 - المجموعة الثانية : فئران مصابون بالسكري تم علاجهم بالانسولين فقط.
 - المجموعة الثالثة : فئران مصابون بالسكري تم علاجهم بالانسولين وتعريض الجرح لاشعة الليزر المرئي ٦٥٠ nm , 150mW حتي التئام الجرح.
 - المجموعة الرابعة: : فئران مصابون بالسكري تم علاجهم بالانسولين وتعريض الجرح للموجات فوق صوتية حتي التئام الجرح.
 - المجموعة الخامسة: : فئران مصابون بالسكري تم علاجهم بالانسولين وعلاج الجرح بجرعات يومية من جزيئات الفضة النانومترية حتي التئام الجرح.
 - المجموعة السادسة: : فئران مصابون بالسكري تم علاجهم بالانسولين وتم استخدام الغشاء النانومتري المصنع من اسيتات السليلوز في الغيار للجرح حتي التئام الجرح.

- **المجموعه السابعه:** فئران مصابون بالسكري تم علاجهم بالانسولين وتم استخدام الغشاء النانومتري المصنع من اسيتات السليلوزو المحتوي علي جزيئات الفضة النانومترية المضادة للبكتريا في الغيار للجرح حتي التئام الجرح.

- تم ملاحظة التئام الجروح في كل المجموعات خلال فترة العلاج لقياس معامل الالتئام
- تم اخذ عينات من الجذ في اليوم الرابع والعاشر والخامس عشر لفحصها هيستولوجيا بعد التئام الجروح
- تم اخذ الجذ الملتئم وقياس الخاصيه الميكانيكيه له.

نتائج البحث:

وقد أسفرت الدراسة عن النتائج الآتية:

- 1- التئام الجروح في الفئران المعالجة باستخدام الغشاء النانومتري المصنع من اسيتات السليلوز والمحتوي علي جزيئات الفضة النانومترية المضادة للبكتريا في فترة اقل من باقي المجموعات.
- 2- تكون كميات من الكولاجين في طبقات الجذ للفئران المعالجة باستخدام الغشاء النانومتري المصنع من اسيتات السليلوز والمحتوي علي جزيئات الفضة النانومترية المضادة للبكتريا مما يدل علي التئام الجروح.

ومن هذه النتائج نستنتج ان:

- لا بد من التطوير في مختلف المجالات وخاصة المجالات الطبية لمحاولة التغلب علي الامراض التي يصعب حلها بالطرق القديمة.
- استخدام تقنيات حديثة مثل استخدام تكنولوجيا النانو قد يساعد في حل العديد من المشاكل في المجالات المختلفة مثل المجال الطبي.

تحضير وتوصيف الغشاء النانومتري المحتوي علي جزيئات الفضة النانومترية المضادة
للبيكتريا لإستخدامه في التئام الجروح في الفرنان المصابة بالسكري مقارنة بالطرق الفيزيائية

للعلاج

رسالة علمية

مقدمة إلى معهد البحوث الطبية- جامعة الإسكندرية
ايفاء جزئيا لشروط الحصول على درجة

الدكتوراه

فى

الفيزياء الحيوية الطبية

مقدمة من

مايسة السيد سيداحمد مصطفى

بكالوريوس العلوم (كيمياء حيوية خاصة ٢٠٠٧)

جامعة الاسكندرية

ماجستير في الفيزياء الحيوية الطبية

معهد البحوث الطبية

جامعة الإسكندرية

٢٠١١

معهد البحوث الطبية

جامعة الإسكندرية

٢٠١٥

تحضير وتوصيف الغشاء النانومتري المحتوي علي جزيئات الفضة النانومترية المضادة للبكتريا
لإستخدامه في التئام الجروح في الفئران المصابة بالسكري مقارنة بالطرق الفيزيائية للعلاج

مقدمة من

مايسة السيد سيداحمد مصطفى

ماجستير في الفيزياء الحيوية الطبية
معهد البحوث الطبية
جامعة الإسكندرية
٢٠١١

للحصول على درجة

الدكتوراه

في

الفيزياء الحيوية الطبية

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