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DEVELOPMENT AND *IN-VITRO* EVALUATION OF *CENTELLA ASIATICA* AND SILVER SOLUTION COATED SUTURES FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT: The research aim is to develop and evaluate *Centella asiatica* Chloroform extract and silver-coated suture against the most common organisms that cause surgical site infections (SSI) by *in-vitro* methods. This prospective study is to coat the *Centella asiatica* (CA) extract to the suture along with the silver nitrate solution. In this study, a suture filament was formulated with Poly Lactic Glycolic Acid (PLGA) (50:50 ratio). A formulated filament was coated with *Centella asiatica* extract, and its property was compared with the marketed future. After minor modifications, the marketed suture was coated with *Centella asiatica* extract. Antibacterial efficacy of *Centella asiatica* extract coated suture filaments with and without silver nitrate solution were evaluated against gram-positive and gram-negative bacteria. Chloroform extract of *Centella asiatica* powder was effective against gram positive and negative bacteria compared to crude powder. Particle size and Zeta potential of the CA powder extract with and without silver nitrate at the end of 3 h was found to be 676.9 and 140.1nm and 0.295 1.45mv of Zeta potential, respectively. The results confirm the addition of silver nitrate reduces the particle size and alters the zeta potential Monofilament PLGA suture didn't show any significance in antibacterial activity against *S. aureus* compared to CA with PLGA and $AgNO_3$. When comparing the antibacterial activity of chloroform extract of CA with PLGA and CA Chloroform Extract, the significant activity ($P < 0.05$) was observed in chloroform extract of CA towards *S. aureus* compared to *E. coli.* Prepared PLGA mono filament shows higher antibacterial action compared to multi commercial filament pre-processed marketed l sutures.

INTRODUCTION: Surgical site infections (SSIs) are most common in the post operated surgical areas, and most are restricted at the incision site [1, 2]. It has been reported, most commonly, gram-positive bacteria (*Staphylococcus aureus)* affect the surface of surgical implanted surfaces and sutures, and it is responsible for 23% of SSIs [1, 3]. SSIs affects patient mortality also it affects the high medical cost for society [4].

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It is destructing this source of infection lead to greatly reduce the incidence of SSIs [5]. Coating the surface of the medical device is one proposition, such as sutures with antibacterial agents. This prevents the bacterial colonize on the surface of suture material without affecting the mechanical properties of the suture [6].

Poly (lactic-co-glycolic) acid (PLGA) concerned significant interest as a base material for various biomedical applications such as high bio-compatibility; approved by U. S. Food and Drug Administration (FDA) for clinical practice in humans; tailored biodegradation rate based on molecular weight of copolymer ratios; latent qualities of alter the surface properties to achieve better interaction with biological materials [7].

The commonly used biodegradable synthetic polymers for three-dimensional (3D) scaffolds in tissue engineering are saturated poly (α-hydroxy esters), including poly (lactic-co-glycolide) (PLGA) copolymers [8, 9]. The chemical properties of these polymers allow hydrolytic degradation through de-esterification. When it gets degraded, the monomeric components of each polymer are removed by natural pathways. PGA is converted to metabolites or eliminated and PLA can be cleared by the tricarboxylic acid cycle. Due to these properties, PLA and PGA have been used in biomedical products and devices, such as degradable sutures [10]. *Centella asiatica* belonging to the family Umbelliferae is a commonly known medicinal plant as an antimicrobial. *C. asiatica* is claimed to possess a wide range of pharmacological effects, being used for human wound healing, mental and neurological disorders, atherosclerosis, fungicidal, antibacterial, antioxidant and anticancer purposes also useful in the treatment of inflammations. Various chemical constituents are reported in *Centella asiatica* like asiaticoside, madecassoide, made cassic acid, asiatic acid, glucose, rhamnose, terpenoids [11, 12]. *Centella asiatica* decreases inflammation, decreases blood pressure in veins, and increases collagen production, which is important for wound healing. *Centella asiatica* chloroform extract has been reported for the widest zone of inhibition [13]. Chavicol, a phenolic compound present in *Centella asiatica* leaf, is a powerful reducing agent against metallic ion reduction [14]. In the current study, we have developed and evaluated suture coated with *Centella asiatica* chloroform extract powder with and without silver nitrate solution against gram-positive and gram-negative bacteria by *in-vitro* methods.

MATERIALS AND METHODS:

Materials: *Centella asiatica* powders were purchased from Om Sakthi Sree Vensangu Nilayam Coimbatore, Tamil Nadu. Poly [(lactic-co-glycolic acid) PLGA (50/50)], Silver Nitrate ($AgNO_3$), and Chloroform were purchased from Sigma Aldrich, USA.

Preparation of *Centella asiatica* extract: About 10g of powder was placed in 100 ml of organic solvent [Chloroform ($CHCL_3$)] in a conical flask, plugged with cotton; the mixture was kept in an orbital shaker at 180 to 200 rpm for 24 h. After 24 h the mixture was filtered through 4 layers of muslin cloth. The filtrate was kept for 24 h to form a concentrated extract at room temperature than used for further studies.

Suture Filament Preparation: The filament was formulated using 40% w/v of PLGA (50:50) ratio by dissolving 2 gm of PLGA in 5 ml of chloroform. The prepared formulation was passed slowly into a water bath extruder through 5 ml syringe at room temperature. Then the prepared polymer layer was converted into a filament by holding with a glass rod to form a suture filament.

Coating Extract to the Prepared PLGA Filament Monofilament: Accurately 100 mg of *Centella asiatica* extract was taken and dissolved in 1 ml of chloroform to form the coating slurry for the first formulation. Then 100 mg of *Centella asiatica* extract and 2 milliMolar (mM) concentration of $AgNO_3$ was also taken and dissolved in 1 ml of chloroform to form the coating slurry for the second formulation. Separately prepared filament suture of 2 cm length was dipped into the separate coating slurry for 2 min. The suture dipped into the coating slurry was kept drying for 3 h at room temperature. Similarly, after preliminary processing, the multifilament marketed l suture was coated using Centella asiatica extract with and without silver nitrate.

Preparation of Suture Filament Together with PLGA and Centella *asiatica* Extract: *Centella asiatica* extract and PLGA 1:10 ratio (drug and polymer) were dissolved in chloroform. It was kept in a magnetic stirrer to form a viscous solution, and the viscous solution was loaded in a 5 ml syringe. The prepared (*Centella asiatica* extract and 40% PLGA in ratio of 1:10) formulation was passed slowly into a water bath extruder through a syringe at room temperature. Then the prepared polymer layer was converted into a filament by holding with a glass rod to form a suture filament. Then the filament was kept for drying at room temperature 48 h.

Characterization: The two formulation of *Centella asiatica* powder extract with and without silver nitrate solution was measured to check the particle size and zeta potential PDI (Poly Dispersity

Index) of silver ions in normal and reduced state using glass cuvette. Readings were recorded using the dynamic light scattering (DLS) technique using Zeta Sizer. (Nano ZS 90, Malvern Instruments, United Kingdom).

Morphology: Scanning electron microscope (SEM) images were taken at 40kv voltage and 100 x magnification to analyze the surface morphology of the formulated filament suture (SEM Model LEICA stereo scan 440).

Antibacterial Activity Studies:

Subculture Preparation: Gram-positive organism *S. aureus* and gram-negative organism *E. coli* were selected as testing organisms. The assessments were performed according to the antibacterial activity evaluation standard (ISO 20645:2004). The subcultures of *Staphylococcus aureus* and *E. coli* were developed by preparing a broth medium composed of 0.25 g of peptone and 0.125 g of NaCl in 25 ml of water. Prepared 25 ml of broth was separated into 4 test tubes (2 for *S. aureus* and 2 for *E. coli*) and it kept in an autoclave for 20 mins at 121°C & 15 lbs. At the optimum temperature, each test tube was inoculated with loop full of microorganisms (2 for *S. aureus* and 2 for *E. coli*). Then it was incubated at 37° C for 24 h.

Qualitative Assay Agar Diffusion Plate Test: The zone of inhibition study was carried out by using the Muller Hinton agar medium. Two conical flasks were taken, and 20 ml of nutrient agar solutions were prepared 0.76g of Muller Hinton agar in 20 ml water for each conical flask. The petri plates, agar medium, and all other requirements were kept in the autoclave for moist heat sterilization. The study accompanies 2 plates, in which each plate were segmented into two blocks. The aseptic room was treated with UV radiation for 20 min. The laminar airflow chamber's floor was wiped by using ethanol. After the sterilization process, the plates and conical flask containing agar medium were taken into an aseptic room. To each conical flask, 0.5 ml of subcultures were added by using a micropipette. The agar medium was transferred to the petri plates and kept for solidification (pour plate method).

Antimicrobial Activity Evaluation of *Centella asiatica* Extract with and without Silver Nitrate: In the prepared petriplate 4 wells were created around 8 mm in diameter using a mechanical borer. The zone of inhibition was measured for CA raw powder, Chloroform extract of CA, Chloroform Extract of CA with PLGA, chloroform extract of CA with AgNo3 after pouring the combinations into the well. Plates were incubated for 24 h at 37°C.

Statistical Analysis: The antibacterial zone of inhibition measurement results was analyzed. The data's were evaluated by one-way analysis of variance (ANOVA) and student 't' test. The results were considered statistically significant when $p<0.05$.

RESULTS AND DISCUSSION:

Formulation of Suture Filament: Initially, two Concentrations 2% and 4% of PLGA solution were prepared using chloroform as a solvent and subjected for electro-spinning. Suture filament was not obtained by electro spinning technique. Hence, the extrusion technique was followed for suture formulation with 40% PLGA (50; 50).

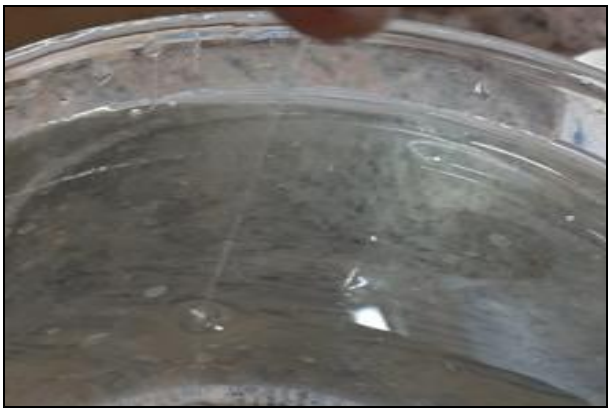

FIG. 1: 2% FORMULATION

FIG. 2: 4% FORMULATION

The above-mentioned 4% PLGA solution was also passed through electro-spinning equipment, but the mat was not formed; only sparkles were seen **Fig. 3** and **Fig. 4** shows the 40%w/v PLGA filament which was prepared by using a water bath extruder.

Fig. 5 shows the *Centella asiatica* chloroform extract and PLGA polymer fused filament which was prepared using a water bath extruder. The results of formulation show the increased quantities of polymer 40% w/v produces comparatively thick filament.

FIG. 3: ELECTRO-SPINNING FORMULATION OF 4% PLGA SOLUTION

FIG. 4: 40 % W/V PLGA FILAMENT WHICH WAS PREPARED BY USING WATER BATH EXTRUDER

FIG. 5: CENTELLA ASIATICA CHLOROFORM EXTRACT AND PLGA POLYMER FUSED FILAMENT PREPARED USING WATER BATH EXTRUDER

Particle Size and Zeta Potential:

Partile size and Zeta Potential of *Centella asiatica:* chloroform extract powder dissolved in chloroform was found to be 177 nm at 10 min with zeta potential value 1.06 Mv with PDI value 0.43, but after 3 h the size was found to be 140 nm and the zeta potential was 1.45 MV with PDI 0.2. This confirms the powder particles' size, zeta potential, and PDI varies with time on the contact of the powder with solvent. But the Chloroform extract powder with silver nitrate solution shows increased particle size (1522 nm) and reduced zeta potential (0.256 Mv) at 10 min. But after 3 h, particle size got reduced to 676.9 nm, and there is not much change in the zeta potential **Table 1.**

TABLE 1: PARTICLE SIZE, PDI AND ZETA POTENTIAL CHARACTERIZATION OF FORMULATIONS

Sl. no.	Formulation	Time	Particle size (nm)	PDI	Zeta potential (Mv)
1	Chloroform solution of *Centella asiatica* extract	10 min	177	0.436	1.06
		3 h	140.1	0.224	1.45
2	Chloroform solution of *Centella asiatica* extract with silver nitrate solution	10 min	1522	0.427	0.256
		3 h	676.9	0.255	0.295

Morphology: Surface morphology of suture formulations was carried out using SEM (Scanning electron microscopy. The naked surgical PLGA suture filament found to have smooth surface **Fig. 6A** with the diameter 37 micron and 50 micron in PLGA filament coated with *Centella asiatica* and $AgNO_3$ due to more adsorption **Fig. 6B**.

These results indicate that hydrophobic PLGA can deposit *Centella asiatica* on its surface without any surface modification.

Pre-processed multi filament marketed suture with improper coating with *Centella asiatica* with the diameter 20-micron **Fig. 6C.**

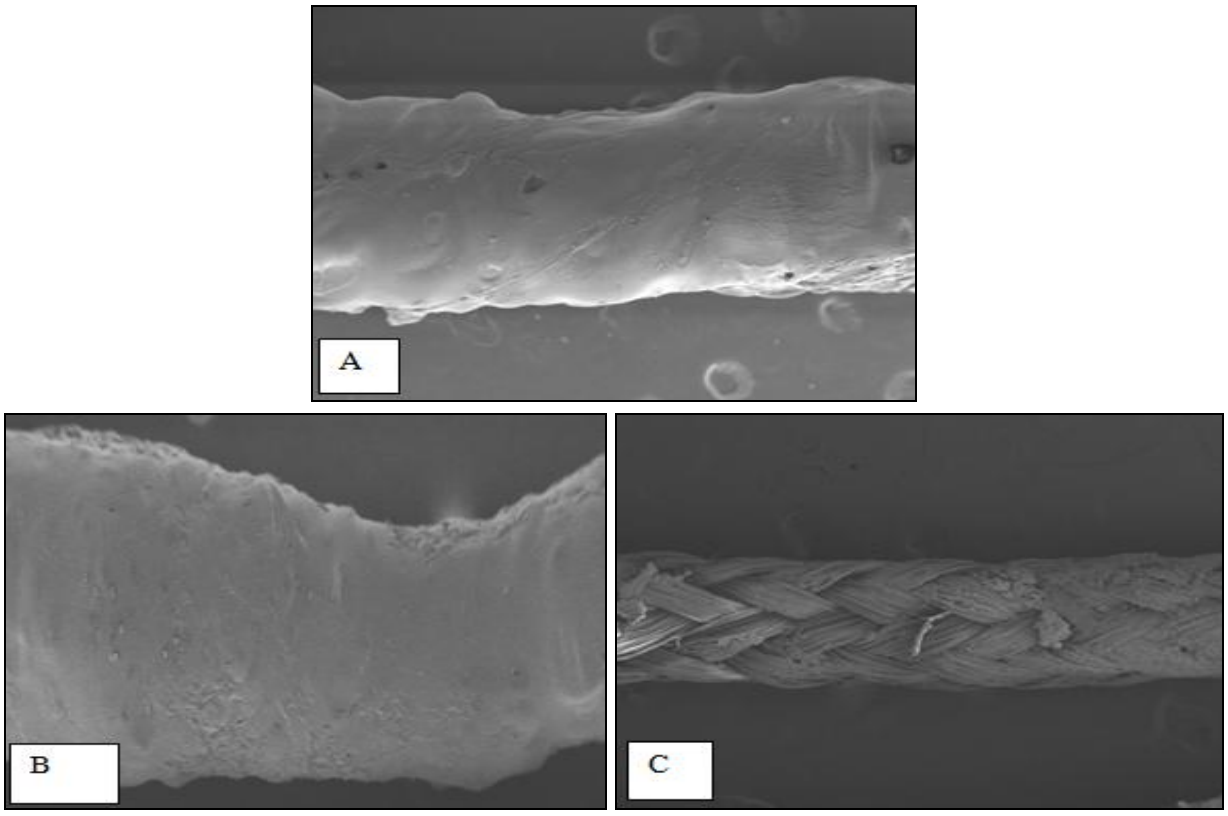

FIG. 6: SEM IMAGES A, PLGA FILAMENT, B, PLGA FILAMENT COATED WITH *CENTELLA ASIATICA* AND $AGNO_3$, C, PRE-PROCESSED MARKETED SUTURE COATED WITH *CENTELLA ASIATICA*

Antimicrobial Studies using Well Diffusion Method: The *Centella asiatica* chloroform extract was tested for an antimicrobial property with and without silver nitrate by well diffusion method. The prepared *Centella asiatica* extract with chloroform $CHCl_3$ produces the effective zone of inhibition on the tested clinical isolated microorganism *i.e. Staphylococcus aureus and E. coli.* The results are tabulated in **Table 2.**

TABLE 2: ANTIMICROBIAL ACTIVITY OF DIFFERENT COMBINATIONS

Group Number	Description	Zone diameter obtained for *Staphylococcus aureus*	Zone diameter obtained for *E coli*
1	*Centella asiatica* raw powder dissolved in chloroform	0.7 cm	0.5cm
2	Chloroform Extract of CA dissolved in chloroform	1 cm	1cm
3	Chloroform Extract of CA dissolved in chloroform with PLGA	0.5cm	0.7cm
4	Chloroform Extract of CA dissolved in chloroform with AgNO3	0.4cm	0.5cm

CA – *Centella asiatica* PLGA - Poly [(lactic -co-glycolic acid) $AGNO_3$– silver nitrate solution.

Chloroform extract of *Centella asiatica* powder was effective against gram-positive and negative bacteria compared to raw powder **Table 2**. It shows significant activity ($P < 0.05$) towards E. *coli* compared to *S. aureus* (Group 1 and 2). When comparing the antibacterial activity of chloroform extract of CA with PLGA and CA Chloroform Extract, the significant activity ($P < 0.05$) was observed in chloroform extract of CA towards *S. aureus* compared to *E. coli* (Group 2 and 3). The antibacterial activity comparison of PLGA with chloroform extract of CA and chloroform extract of CA with $AgNO_3$ didn't show any significance in activity between Gram-positive and gram-negative bacteria (Group 3 and 4).

Antibacterial Activity Studies for Coated and Prepared Sutures:

Zone of Inhibition (*S. Aureus*): Fig. 7A (CA+ PLGA) Antimicrobial effect of *Centella asiatica* coated with prepared PLGA monofilament suture. **Fig. 7B** (CA+ PLGA+$AgNO_3$) Antimicrobial effect of *Centella asiatica* coated with prepared PLGA monofilament suture with 1mM silver nitrate. **Fig. 8A** (CA+VP) Antimicrobial effect of *Centella asiatica* coated with multifilament pre-processed marketed suture. **Fig. 8B** (CA+VP+AgNO3) Antimicrobial effect of *Centella asiatica* coated with multifilament pre-processed marketed suture with 1mM silver nitrate.

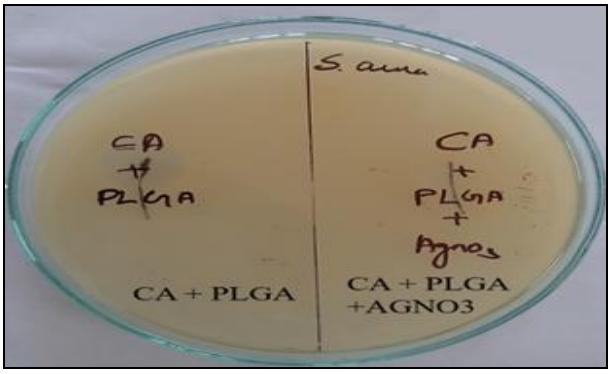

FIG. 7: ZOI FOR FORMULATION OF 7A AND 7B

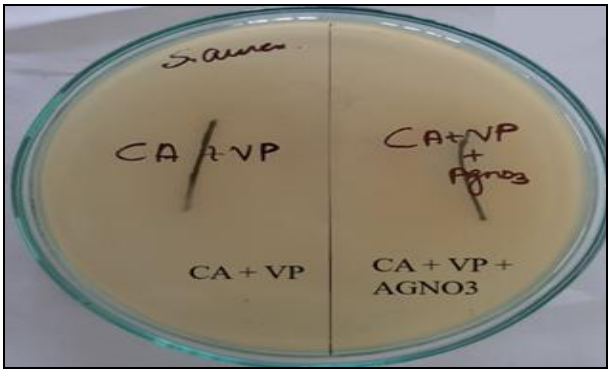

FIG. 8: ZOI FOR FORMULATION OF 8A AND 8B

TABLE 3: ZONE OF INHIBITION VALUES AGAINST *S. AUREUS*

Formulations	Zone of inhibition diameter for *S. aureus* at the end of 24 h
Monofilament suture with CA extract along with PLGA	1.3 cm
Mono filament suture with CA extract, PLGA and $AgNO_3$	0.5 cm
Multifilament processed VP with CA extract	0.2cm
Multifilament processed VP with CA extract and $AgNO_3$	1.1 cm

CA – *Centella asiatica* PLGA – Poly (lactic-co-glycolic acid) VP –marketed suture pre-processed.

Monofilament suture with PLGA shows a significantly ($P<0.05$) high zone of inhibition against *Staphylococcus aureus* compared to CA with PLGA and AGNO[3]. *Centella asiatica* has been reported in literature [59] for its reducing agent property. Chavicol present in *Centella asiaticais* a phenolic compound and powerful reducing agent against metallic ion reduction [59]. $AgNO_3$ solution in the presence of CA should have converted to reduce silver solution and it should have got adsorbed on PLGA. The release of CA from PLGA adsorbed reduced silver solution may be slower, leading to less zone of inhibition **Table 3.** The study results also reveal that prepared PLGA mono filament shows higher antibacterial action than marketed multi filament marketed sutures. This may be due to the coating of the prepared slurry to the uncoated prepared suture filament. But when comparing CA with VP higher zone of inhibition with significance ($P <0.05$) was observed in CA with VP and $AgNO_3$. The presence of CA along with reduced silver solution should have produced synergic antibacterial activity. Similarly, comparison of monofilament suture coated with CA, PLGA and $AgNO_3$ results with multifilament suture coated with CA, VP, and $AgNO_3$ confirm multifilament shows significant ($P <0.05$) antibacterial activity.

Zone of Inhibition (*E. coli*): Fig. 9A (CA+VP) Antimicrobial effect of *Centella asiatica* coated marketed suture which inhibiting *E. coli*.

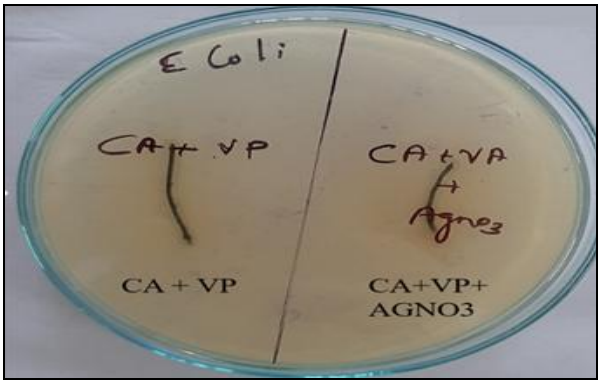

FIG. 9: ZOI FOR FORMULATION OF 9A AND 9B

Fig. 9B: (CA+VP+$AgNO_3$) Antimicrobial effect of *Centella asiatica* coated pre-processed marketed suture which Inhibiting *E. coli.* This study results from **Fig. 9** confirm that coating *Centella asiatica* in marketed pre-processed commercial multifilament suture (VP) cannot produce a good antibacterial effect against gram-negative microorganisms. It may be due to less adsorption of *Centella asiatica* extract and silver ions solution. No statistical difference was observed between (CA with VP) and (CA with VP and $AgNO_3$).

TABLE 4: ZONE OF INHIBITION VALUES AGAINST *E. COLI*

Groups	Figure No	Formulations	Zone of inhibition diameter for *E. coli* after 24 h
1	9A	CA with VP	0.2 cm
2	9B	CA with VP and $AgNO_3$	0.18 cm

CA – *Centella asiatica*, VP –Marketed pre-processed suture.

CONCLUSION: The present study revealed that the coating of Centella asiatica chloroform extract powder on prepared PLGA filament shows higher antibacterial action than marketed pre-processed sutures.

This novel *Centella asiatica* coated herbal suture may be an alternative to already established antimicrobial sutures. It may be useful to reduce the risk of surgical site infections caused by susceptible bacteria.

FUTURE DIRECTION: In future perspective if prepared monofilament were braided to a multifilament using braiding equipment, for improving the tensile property of the prepared filament

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