Biofilm formation in uropathogenic strains of *Proteus mirabilis* and their susceptibility to poly(epsilon-lysine) dendron

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**ABSTRACT**

*Proteus mirabilis* is the main aetiologi cal bacteria in catheter-associated urinary tract infections (CAUTI). The ability of *P. mirabilis* to form biofilms plays an important role in the pathogenesis of CAUTI and there are currently no effective antibiotic therapies. Hence, the aim of this study was to detect the growth and biofilm forming ability of *P. mirabilis* human isolates on 96-well microtitre plates, and study the antimicrobial effect of poly(epsilon-lysine) dendron molecules on the *P. mirabilis* biofilms. Eight strains isolated from human urinary tract infections and verified as *P. mirabilis*, were obtained from the Royal Sussex County Hospital, Brighton, UK. The biofilm forming potential of the selected strains was investigated over 24 hours in 96-well microtitre plates. A clinically relevant concentration of bacteria (10^7 CFU/ml) was incubated in triplicate in Luria-Berthani growth medium. Analysis of the biofilms was performed using crystal violet staining and microscopic observations. *P. mirabilis* strains with the highest biofilm forming ability were selected for their antimicrobial study. Poly(epsilon-lysine) dendron (generation three) were synthesised via solid-phase peptide synthesis, using Fmoc-protected amino acids and characterised with mass spectrometry and high-performance liquid chromatography. The dendron (1–0.25 mg/ml) was then co-incubated with *P. mirabilis* bacteria for 24 hours at 37°C, and the corresponding effect on biofilm formation was assessed as above. Statistical analyses were performed using analysis of variance (ANOVA), with statistical significance defined as P < 0.05. All isolates formed biofilms on polystyrene surfaces. The dendron decreased *P. mirabilis* biofilm formation predominantly (>70 %), by decreasing exopolysaccharide production in a dose-dependent manner. These findings suggest that poly(epsilon-lysine) dendron could have a role in the design of alternative antimicrobial agents for control of *P. mirabilis* biofilm-related infections. Future studies will focus on the dendron’s ability to eradicate pre-formed biofilms.

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**Keywords:** dendron; poly(epsilon-lysine); *Proteus mirabilis*; biofilm; urinary tract infection.

**1. INTRODUCTION**

Antibiotic resistance is currently a growing global crisis that continually threatens the successful treatment of bacterial infections [1]. In line with this there is a need for alternatives or novel combinatorial therapeutic strategies that could enhance antibiotic action. The problem of antibiotic resistance is heightened where biofilms occur [2]. Biofilms are sessile aggregates of microorganisms, encased in a self-produced extracellular matrix (ECM) [3-5], and are problematic on medical device surfaces [5], in wound infections [6,7] and industrial
operations (bio-deterioration) [8]. A key step in the formation of biofilms is the production of exopolysaccharides (EPS). The EPS confers significant structural stability to the biofilm, and is a component of the ECM [9]. Hence, one of the objectives of this study was to evaluate the biofilm forming ability of clinical bacterial isolates. With a focus on Proteus mirabilis bacteria obtained from human urine samples. A quantitative microtitre plate assay was used to compare biofilm formation between strains. Since P. mirabilis is the most frequently isolated pathogen in long term catheterised patients [10], characterising its biofilm forming ability will further develop insights into differences that exist between strains of P. mirabilis, in addition to highlighting how these differences could impact treatment success of novel therapeutic strategies.

Since the extracellular matrix provides bacteria protection from antibiotic action, alternatives to antibiotics have been sought such as bacteriophage [11], lytic enzymes [12], antimicrobial metals [13,14] and nano-molecules such as dendrimers [15,16]. Dendrimers are mono-disperse, hyperbranched molecules, which can be synthesised from a variety of molecules such as organic, saccharides or amino acids, and are easily modifiable to promote biological action. They have been mostly implemented in the search for anti-cancer therapies and imaging [17,18]. However, their usefulness as anti-biofilm molecules is currently developing, as at high generations, bactericidal activity is reported [19]. Based on this and the reported antibacterial action of randomly branched epsilon-poly-L-lysine polymers [20,21], dendrons (a sub-group of dendritic polymers) of poly(epsilon-lysine) were synthesised in order to assess their ability to prevent biofilm formation.

The aim of the present study was to describe an innovative strategy to combat biofilm formation. The strategy involved using generation three (Gen3) poly(epsilon-lysine) dendrons (Gen3K), which have a different structure to the randomly branched poly-L-lysine polymers, and have the potential for downstream modification of its reactive branched peripheral ends. The dendron was synthesised by solid-phase peptide synthesis. The anti-biofilm efficacy of this nano-molecule was evaluated on Gram-negative Proteus mirabilis. Structural deformations and insights in the mechanism of action were also assessed.

To our knowledge, this is the first report that describes the effects of a generation three poly(epsilon-lysine) dendron on P. mirabilis biofilms.

2. MATERIAL AND METHODS

2.1 Synthesis and Characterisation of poly(epsilon-lysine) dendron

The Gen3K dendrons were produced from a manual solid phase peptide synthesis method using Fmoc (9-fluorenylethoxycarbonyl)-protected amino acids. Fmoc rink amide linker, Na, Nε-Bis Fmoc-L-lysine and TentaGel S-NH₂ resin (bead size 90 µm) were supplied by Iris Biotech, GmbH. All solvents were of analytical quality. N, N-diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA), piperidine and trisopropyl silane (TIPS) were purchased from Sigma Aldrich. Diethyl ether, methanol, acetonitrile, dichloromethane and N, N- dimethylformamide (anhydrous solvent synthesis grade) were obtained from Thermo Fisher Scientific. 2-(1H-benzo[d]azol-1-yl)-1,1,3,3-tetramethylyuronium hexafluorophosphate (HBTU) was purchased from Novabiochem. Distilled water of 15 mΩ quality was used when required.

Synthesis was performed in 10 ml polypropylene fritted syringes, in line with the Fmoc-based synthesis method used by Meikle et al. [22]. The reaction was carried out in a stepwise manner. Firstly, 0.5 g TentaGel S-NH₂ resin was allowed to swell for 15 minutes (min) in 5 ml N, N-dimethylformamide (DMF), after which, the solvent was expelled from the syringe. The Fmoc-rink amide linker was then attached to the resin, by addition of 0.4 mmol linker and 0.4 mmol HBTU dissolved in 140 µl of DIPEA and 3 ml DMF. The reaction was left to occur at room temperature for 30 min, followed by expulsion of the solvent and triple washing steps with 3 ml DMF. In order to attach the lysine amino acids, a 4x molar excess (0.4 mmol) of Fmoc-protected amino acid with 0.4 mmol HBTU dissolved in 140 µl of DIPEA and 3 ml DMF. Fmoc-protecting groups were removed in a deprotection step using 20 % v/v piperidine in DMF, to allow peptide bond formation between amine (-NH₂) and carboxyl (-COOH) groups. After each generation of lysine had been synthesised, this was always followed by five DMF washing steps, to remove unreacted amino acids and waste products. The coupling and deprotection reactions were all conducted for 30 min at room temperature without shaking. The synthesised Gen3K, still attached to the resin was washed eight times each with 5 ml dichloromethane, then methanol and finally with diethyl ether.

To release the synthesised dendron from the resin, cleavage of the final product was carried out using a mixture of TFA/H₂O/TIPS (95: 2.5: 2.5 % v/v) solution for 3 hours (h). After which, the cleaved dendron was collected by separation of the resin from the dendron through

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a glass Pasteur pipette stoppered with glass wool up to a depth of 0.5 cm and clamped over a 50 ml tube containing 20 ml diethyl ether on ice. Over time, the cleaved dendron was filtered through and appeared as a white precipitate in the diethyl ether. The precipitate was collected by centrifugation at 3,500 rpm for 5 min, resuspension in diethyl ether and centrifugation was repeated twice. Afterwards, the crude peptide was allowed to air dry in a fume hood overnight, before being freeze dried (Christ Alpha2-4, UK). A sticky faint yellow solid of Gen3K was obtained.

Characterisation of the dendron was performed using mass spectrometry and high performance liquid chromatography (HPLC). For analysis of Gen3K, the dendron was dissolved at a concentration of 1 mg/ml in methanol and subjected to mass spectrometry (Bruker microTOF). The calculated molecular weight was obtained by using Equation 4.1.

\[ m/z = (MW+nH)/n \] \[ \text{Equation 4.1} \]

For HPLC, the same concentration of dendron used for mass spectrometry, was employed. The HPLC method consisted of timed gradients of acetonitrile and water: first 15 min 95 % water (A) and 5 % acetonitrile (B), to 20 % A and 80 % B, then hold for 3 min at 20 % A and 80 % B, followed by 1 min 0 % A and 100 % B, and finally return to the original solvent settings after 1 min 95 % A and 5 % B, 20 µl injection volume, at 1 ml/min flow rate, with UV detection wavelength at 223 nm. The HPLC separation of the peptide was performed on a column of size 150 x 4.60mm, Luna 3u C18 100Å, Phenomenex, UK) at 25 °C (Column chiller Model 7955, Jones Chromatography, UK) using an HPLC instrument (Waters™ 2487 dual absorbance detector, and Waters™ 717plus autosampler, UK). Resulting chromatograms were recorded on UV detector (SPO-6A, Shimadzu, UK), and analysed by Total Chrom-TC Navigator software

2.2 Bacteriological studies

2.2.1 Bacterial isolates, media and growth conditions

Clinical strains of *P. mirabilis* from urine were obtained from the diagnostic microbiology laboratory of the Royal Sussex County Hospital, Brighton, UK. Bacterial strains were cultured in tryptone soya broth (TSB), with aeration (120 rpm) at 37°C, cultures were stored in 10 % v/v glycerol at -80°C

2.2.2 Bacterial identification

Bacterial identification was confirmed using the bioMérieux API® 20E testing kit according to the manufacturer’s instructions, and strains were characterised by the Dienes test. The Dienes test is a simple method for identification of unique strains within the same species. Basically, two or more strains are spotted onto a swarming agar plate such as TSA. If the two strains are unrelated, a Dienes demarcation line develops, but if the strains are the same, they swarm over each other and no demarcation line is observed [23]. For the Dienes test, *P. mirabilis* were cultured at 37°C for 18 h, then 5 µl of each overnight culture was dropped onto a 1.5 % TSA agar plate, the spots were allowed to dry, each plate was inverted and incubated at 37°C for 18 h. The experimental design was prepared to allow all strains to be paired against each other.

2.2.3 Assessment of biofilm forming ability

The microtitre plate model was used for assessment of biofilm forming ability by *P. mirabilis* strains. Each strain which had been incubated in TSB at 37°C for 18 h was centrifuged at 7,000 x g for 5 min. The bacterial pellets were resuspended in phosphate buffered saline and standardised to an optical density (OD) of 1 at wavelength 600 nm. Each bacterial strain was then further diluted 1:50 in Luria Berthani (LB) broth (Oxoid, UK) to obtain a concentration of 1 x 10⁷ (CFU/ml) bacterial concentration which lies above the most common diagnostic threshold (10⁶ CFU/ml) for urinary tract infections (UTI) [24]. To each one of the triplicate wells of the non-tissue culture treated, flat bottomed microtitre 96-well plate (Corning, Inc.), 0.1ml of this bacterial suspension was added. The plates were incubated statically at 37°C for 24 h to allow for biofilm formation. For negative controls, at least three wells of each microtitre plate were handled in the same way, containing only LB broth growth medium.

After growth of biofilm, the planktonic cells were discarded and each well rinsed with sterile reverse osmosis water. Each well was allowed to dry before the addition of 0.2 ml 0.1 % v/v crystal violet (CV). Staining was performed for 20 min, afterwards, each well was rinsed as previously described and tapped dry on a paper towel. To solubilise the adhered stain, 95 % v/v ethanol at 600 nm was used. For classification of biofilm forming ability, *P. mirabilis* strains were placed into four categories: none biofilm former, weak, moderate and dense (Table 1), based on the method used by Kwiecińska-Pirog et al [25]. Classification was based on the measured OD of the ethanol supernatant following solubilisation of adhered stains. From these results, the densest biofilm formers were selected to analyse the ability of poly(epsilon-lysine) dendron to prevent biofilm formation.
Table 1. Classification of strains based on biofilm production

<table>
<thead>
<tr>
<th>Range of optical density (OD)</th>
<th>Classification of biofilm production</th>
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</thead>
<tbody>
<tr>
<td>OD ≤ ODc*</td>
<td>None</td>
</tr>
<tr>
<td>ODc &lt; OD ≤ (2 × ODc)</td>
<td>Weak</td>
</tr>
<tr>
<td>(2 × ODc) &lt; OD ≤ (4 × ODc)</td>
<td>Moderate</td>
</tr>
<tr>
<td>(4 × ODc) &lt; OD</td>
<td>Dense</td>
</tr>
</tbody>
</table>

*ODc is the calculated optical density cut-off value: OD of the negative control + 3xstandard deviation.

2.2.4 Biofilm inhibition assay

_P. mirabilis_ UTI strains (Pm289 and Pm425) with high biofilm forming ability were selected for use. In 96-well microtitre plates, the bacterial cells (1 x 10⁷ CFU/ml) were mixed with varying concentrations of the dendron (0.25 - 1 mg/ml), and incubated statically at 37°C for 24 h. Triplicate wells were incubated for each treatment dose, and two independent replicates were performed on two different days. The growth of cells was measured at OD₆₀₀, and biofilm density was evaluated after 24 h treatment. Following crystal violet staining, biofilm density of treated cells was compared to the untreated controls. Microscopic evaluation of biofilm prevention was also performed by inverted light microscopy.

2.2.5 Microscopy

To observe physical structure of the biofilms, light microscopy using an Axiowert 25 inverted microscope (Zeiss, USA) was performed. This allowed qualitative assessment of the adherent bacteria and extracellular matrix formation. The images were obtained using 32x objective lens, and captured using an attached live camera (QIClick Digital CCD Camera, QIMAGING, USA).

2.3 Statistical analysis

All graphical evaluations were made using MS Excel (Microsoft systems). Analysis of variance (ANOVA) was used to evaluate significant differences, with the Minitab® 17 software. P value < 0.05 was used as the significance level.

3. RESULTS

3.1 Synthesis and characterisation of generation three poly(epsilon-lysine) dendrons (Gen3K)

Schematic representation of Gen3K shows the structure possesses 16 free amines at the periphery and a core of carboxylic acid functional group, with molecular formulae C₉₀H₁₈₂N₃₀O₁₆ (Fig. 1). The calculated molecular weight (1939.53) was 99.94% of the theoretical molecular weight of 1940.64 Da, hence the synthesised molecule had a molecular weight close to the expected value. The HPLC chromatogram was obtained at 223 nm with a detected retention time of approximately 11.2 minutes (Fig. 1B). A single peak was observed with no other peaks apart from the solvent front.

3.2 Bacterial Isolation and Identification

All Proteus isolates were identified as _P. mirabilis_. With confirmation as different strains based on the Dienes test.

3.3 Variability in biofilm formation within _P. mirabilis_ strains

In the screening of _P. mirabilis_ 24 h biofilm formation, biofilm classification was within the moderate to dense classification. Four strains (50%) produced biofilms that were significantly denser than the blank (no bacteria) control. Although differences in biofilm density were observed between strains, statistically significant differences between these strains were not observed (One-way ANOVA, p > 0.05) (Figure 2).

Highest density values were observed for strains Pm289 and Pm425 (mean 0.78 and 0.79, respectively) which were approximately 39 times the OD₆₀₀ at start of growth. Only two strains Pm551 and Pm160 showed medium biofilm density with mean OD₆₀₀ values of 0.35 and 0.33 respectively. Hence, strains Pm289 and Pm425 were selected being the densest biofilm formers, to evaluate the anti-biofilm activity of Gen3K dendron.

3.4 Generation three poly(epsilon-lysine) dendron (Gen3K) prevent _Proteus mirabilis_ bacterial biofilm formation

The antimicrobial effect of Gen3K on _P. mirabilis_ biofilm formation was assessed by the microtitre plate assay. Here, the formation of biofilms was revealed by crystal violet staining of cells and biofilm-associated structures.
Fig. 1. Synthesis, structure and characterisation of poly(epsilon-lysine) dendrons (Gen3K).
(A) Overview of Gen3K synthesis based on synthesis condition: (1) 4x molar excess Fmoc Lys(Fmoc)-OH, HBTU, DMF, DIPEA, 30 min (coupling step per lysine); Fmoc deprotection after each generation was performed in 20% v/v piperidine (2 min, x3 to give generation 0 and 1; 2 min, x3 and 30 min once to give generation 2 and 3 (Gen2K and Gen3K)); to yield the final dendron, TFA/TIPS/H₂O at ratio 95:2.5:2.5 % v/v was used. (B) the structure of Gen3K, obtained by using ChemDraw (CambridgeSoft) software, (C) mass spectra of Gen3K and (D) HPLC chromatogram of Gen3K conditions.

Fig. 2. Biofilm formation of *P. mirabilis* strain
Mean biofilm density of different *P. mirabilis* strains in microtitre plates at 37°C represented by turbidity measurements at OD₆₀₀. Bars represent standard error of the mean of three independent replicates. *Biofilm densities that were significantly different from the no host control (p < 0.05).
Fig. 3. *P. mirabilis* biofilm prevention by Gen3K
Biofilm prevention was performed on strains Pm289 and Pm425 with Gen3K added at doses 1 – 0.25 mg/ml, and treated for 24 h. The control was each strain without the addition of Gen3K. The values shown are mean biofilm density with standard error bars of the mean of two independent replicates.

Fig. 4. Microscopic examination of disruption of biofilm structure by Gen3K
The biofilm architectures of *P. mirabilis* strains Pm289 (A-C) and Pm425 (E-G) showed reduced aggregation of bacterial cells and or complete elimination of biofilm-related extracellular matrix after 24 h G3K treatment, compared to their respective controls (D and H). The action of Gen3K was dose dependent, at 1 mg/ml (A,E) the extracellular matrix was completely prevented from forming, and only singular adherent cells were visible, compared to the controls (D,H) which showed aggregated cells encased in the extracellular matrix. At lower concentrations of 0.5 mg/ml (B,F) and 0.25 mg/ml (C,G) the extracellular matrix was still significantly cleared compared to the controls, however some aggregation of cells was observed for strain Pm425, which formed denser biofilms than Pm289. These results correlated with measurement of biofilm density in Figure 3.
The growth media containing bacteria without the Gen3K dendron served as untreated control. Gen3K reduced *P. mirabilis* biofilm formation to significantly lower levels compared to the untreated control (Figure 3). The biofilm preventive effect of Gen3K was remarkable with 80%, 81% and 78% reduction in biofilm density for strain Pm289 at Gen3K doses 1-0.5mg/ml respectively, and for Pm425 the reductions were 77%, 72% and 63% respectively in decreasing order of dosage. With respect to bactericidal activity, the dendron was less effective at killing, showing a maximal 14% cell death at 1 mg/ml for strain Pm289 and 8% cell death at all treatment doses for strain Pm425 after 24h incubation (data not shown).

3.5 Biofilm extracellular matrix eliminated by the poly(epsilon-lysine) dendron (Gen3K)

To characterise the biofilm architecture, microscopic examination of the Gen3K treated and untreated biofilms was performed. A reduction in extracellular matrix was observed at all treatment doses (Fig. 4). The mesh-work of exopolysaccharides composing the extracellular matrix is clearly visible in the controls (Figs. 4D and 4H), with Pm425 being more compact than Pm289.

4. DISCUSSION

Several studies have shown that *P. mirabilis* is an important pathogen in biofilm related infections associated with long-term catheterisation and less commonly in wound infections [26-29]. Current strategies for prevention of catheter associated urinary tract infections are focused on limiting use and duration of catheter in situ [30]. *P. mirabilis* biofilm prevention is important as *P. mirabilis* has a high ability to migrate over solid surfaces via a process called swarming and form biofilms on biomaterials [31], as shown here on polystyrene microtitre well surfaces. All strains implemented in this study were able to form biofilms, although to varying degrees. The biofilm density correlated with the presence of biofilm extracellular matrix (ECM), which is a potent virulence factor in the persistence of biofilm infections. Strain Pm425 produced the densest biofilm compared to other strains, and microscopy examination showed an intense exopolysaccharide component of the ECM. Since biofilms are the usual living mode to prevent antimicrobial attack [32], it was not unusual to see that all strains formed biofilms in vitro. Reports have shown that swarmer cells of *P. mirabilis* can incur up to 30-fold increase in polysaccharide production, so it possible than strain Pm425 is a more effective swarmer compared to Pm289 hence its increased ECM abundance [41,42].

With the global challenge of antibiotic resistance, there is a steer towards the development of novel antimicrobials, as antibiotic susceptibility is impaired even up to a thousand fold in the presence of biofilms [33]. Hence, the main aim of this study was to test whether generation three poly(epsilon-lysine) dendrons could effectively prevent the formation of *P. mirabilis* biofilms. Gen3K was chosen to monitor its therapeutic potential, as dendrimeric molecules have been shown to have a significant antibacterial action in other species [34]. The branched structure of dendrimeric molecules provide increased interaction sites with its target, and can be harnessed for antibiotic loading [35,36]. In addition, the Gen3K molecule synthesised is hydrophilic, with an overall positive charge that could interact with the negatively charged cell wall of Gram negative bacteria, such as *P. mirabilis* [37]. The synthesised molecule was successfully characterised by mass spectrometry and from the HPLC (Fig. 1D), the single prominent peak showed that one type of compound was present, with lack of other prominent peaks, it was interpreted that there were no impurities present in the synthesised compound. Results by Zhang et al. have shown that polycationic properties of chitosan and poly-L-lysine based molecules promoted high anti-biofilm capacity [40], hence at higher doses of Gen3K there was increased anti-biofilm activity.

Our data showed that the dendron (Gen3K) was more effective in prevention of biofilms than in killing of bacterial cells (Figs. 2 and 3). This was the case for all strains tested, with most detriment observed for strain Pm289, however, the differences observed where minimal and not statistically significant. With regards to biofilm prevention, it is proposed that Gen3K may function via interruption of extracellular matrix synthesis either at DNA transcription or translation of associated proteins needed for ECM synthesis, with negligible effect on bacterial growth. This action of ECM clearance by the Gen3K molecule may prove useful in combined therapy. The biofilm extracellular matrix is an important virulence factor in the persistence of biofilm-related infections, as it sequesters bacterial cells from antibiotic attack [38]. Therefore, the ability of Gen3K to eliminate the extracellular matrix could be beneficial in promoting antibiotic access to susceptible bacterial cells. Thereby, increasing antibiotic susceptibility of biofilm embedded bacterial cells. This could overcome resistance of biofilms to antibiotics and aid the control of biofilms in industrial and medical areas. With the presence of bacterial aggregation, via extracellular matrix at lower doses of the dendron, it is possible that the positive charge of the Gen3K molecule may interact with regions of negative charge in the biofilm structure such as the extracellular DNA, negatively charged.
exopolysaccharides and protein moieties of the ECM. Thus limiting its availability to the remaining adherent cells encased in the biofilm [39,40]. Since Pm425, possessed more ECM compared to Pm289, it was expected that the biofilm density reduction would be less effective in Pm425 strain. These observations highlighted the need to microscopically confirm structural defect in the biofilms, and the data obtained (Fig. 4) supported the hypotheses that Gen3K was more effective when levels of ECM were lower.

5. CONCLUSION
This study demonstrated high prevalence of biofilm producers among P. mirabilis uropathogenic strains. The generation three poly(epsilon-lysine) dendron is an active anti-biofilm agent with a dose dependent effect on prevention of biofilms. With a correlation between treatment dose and prevention of extracellular matrix production, this suggests that the dendron may act by impairment of extracellular matrix synthesis. This study has shown that generation three poly(epsilon-lysine) dendron has potential as a novel therapeutic strategy for prevention of Proteus mirabilis biofilm infections. Further investigations will determine the effects induced by this compound on pre-formed biofilms of P. mirabilis. While this study is in its nascent phase of development, further development of this dendron-based technology could eventually lead to anti-biofilm therapies that can be used solely or in combination with antibiotics.

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AUTHOR CONTRIBUTIONS
‘Orode Aniejurengho, Steve Meikle and Matteo Santin’ designed the research. ‘Orode Aniejurengho’ performed research, statistical analysis, and wrote the first draft of the manuscript. ‘Mariagemiliana Dessi, Steve Meikle and Matteo Santin’ revised the manuscript. All authors read and approved the final manuscript.

REFERENCES


