Author **(year)**	**Positive Outcomes**	**Negative Outcomes**	**Strengths**	**Limitations**	**Suggested study improvements**
Liao et al. (2019) (19)	• Auricle stiffness similar to human ear • Fusion of diced cartilage created sufficient pinna strength and flexibility • Fusion of main pinna structures at 4 months • No complications (haematoma, seroma or infection) at implantation site	• Synthetic scaffold (polyamide) induced a cystic-like reaction subcutaneously • Not all structures fused by 4 months • Chondrocytes harvested from healthy tissue with donor site morbidity	• Prospective study • Used compression plates for biomechanical strength testing of pinna • Histological staining used to assess presence of chondrocytes, matrix and type II collagen • Ethical approval	• Animal study • 10 subjects • 12 week end point • One scaffold material tested • Neocartilage weight used as success marker not considering possible calcification or bone formation • Appearance of pinna not assessed (primary outcomes presence of chondrocytes, matrix and strength) • Consider difference in pressure exerted on this subcutaneous dorsal pinna vs tight human pinna skin and shape/size changes overtime • No testing for cartilage calcification or bone formation	• Larger numbers with longer follow up • Consideration of aesthetics • Human application • Implant removal and QPCR analysis for bone markers along with alizarin staining to rule out calcification
Zhou et al. (2018) (22)	• Successfully implanted tissue engineered ear into 5 children • Individualised shape based on unaffected ear • Shape of bioengineered pinna pre-implantation (12 weeks) showed >90% similarity to the original bioscaffold on laser scanning • Pinna contours (helix, triangular fossa, anti-helix and cavum conchae) defined by 9 months • Majority of scaffold PGA fibres degraded during 3 months in-vitro prior to implantation, minimising host response • PCL core in scaffold significantly improved strength of reconstructed pinna in vitro 4-fold	• Reconstructed ear stiff and inflexible 12 months post-operatively (note improved by 24 months with further PCL degradation) • One of the five subjects lacked evidence of cartilage formation 6 months post-operatively • Some surgical implantation methods predispose to graft extrusion	• Prospective human study • Clear primary outcomes of shape, size and cranio-auriculo angle • Detailed step-by-step explanation of the 3 methods used • Ethical approval • SEM used regularly to analyse adherence of chondrocytes to scaffold • Quality control sample of the tissue-engineered cartilage used for assessments • In vitro method enables quality assessment prior to implantation • Shape analysis using 3D laser scanning prior to implantation • Regular post-operative review at 1, 2, 3, 6, 9, 12, 18, 24 and 30 months. • Histological and immunohistochemical analysis of neocartilage post-operatively (tragal biopsies taken at revision surgeries)	• Five subjects • Only one case followed up in detail (others to follow) • Maximum follow up of 2.5 years (other cases ranged 2-18 months), not allowing for complete degradation of scaffold (takes 2-4 years) • Single centre • This method only suitable for grade II or III microtia, where microtia cartilage can be obtained • Tissue expander required for 3 months with psycho-social impact • Split-thickness skin graft from groin required at initial procedure • Subsequent flap/scar revision surgeries required at 6 and 18 months	• More subjects • Longer follow up (min. 5 years allowing complete scaffold degradation) • Use of more mature neocartilaginous grafts recommended, easing surgical handling • Multicentre trial • Standardised surgical method of implantation • Histological analysis including alizarin to rule out calcification/hypertrophy and bone formation pre-implantation • MRI/CT scan to identify possible calcification of implant once in-situ
Zopf et al. (2018) (57)	• Demonstrates the effect of scaffold microarchitecture on cartilage formation. Greater chondrogenicity using regular spherical micropores vs random pore placement within scaffold.	• Not disclosed	• Prospective study • Ethical approval • Clear methods • Primary outcome of chondrogenesis clearly assessed • Histological analysis of auricle following removal	• Animal study • 4 week end point • Study size unknown • No testing for cartilage calcification or bone formation • No strength testing	• Longer duration allowing for shape and size changes • QPCR analysis for bone markers along with alizarin staining to rule out calcification

			• Aesthetics considered as secondary outcome considering auricular contours, dimensions and projection	• Consider difference in pressure exerted on this subcutaneous pinna vs tight human pinna skin and shape/size changes overtime	
Pomerantseva et al. (2016) (58)	• High-quality neocartilage formed by 12 weeks confirmed by GAG, collagen type II and elastin • Superior neocartilage in the group expanded using bFGF-expanded chondrocytes • Enhanced elastin fibre quality in neocartilage formed from bFGF-expanded chondrocytes • Neocartilage quality improved with time from implantation • ≤ 10% dimension change at 20 weeks • No neocartilage resorption from 6-20 weeks	• Some resorption of neocartilage before 6 weeks • Some shrinkage of neocartilage after 20 weeks • Poor elastin formation at 12 weeks in group not expanded with bFGF	• Clear description of scaffold design and in vitro methods • Histological and immunohistochemical analysis with full thickness punch biopsies • Prospective study • Ethical approval	• Animal study • Short follow up (6, 12, 20 weeks) • Punch biopsies used for analysis rather than full size cross sections showing contiguous neocartilage (due to titanium wire) • Scaffold implanted subcutaneously in neck and subject to less pressure than post-auricular placement • No testing for cartilage calcification or bone formation	• Longer follow up allowing for shape and size changes • Implant removal and QPCR analysis for bone markers along with alizarin staining to rule out calcification
Zopf et al. (2015) (59)	• Histology of in vitro constructs showed cartilage-like tissue at end point • Patient-specific auricles using CT scanning and CAD	• Incomplete cartilage fusion at end point (2 months)	• 2 institutions • Ethical approval • Prospective study • Clear description of scaffold design and in vitro methods • Histological analysis and staining of auricles • Scaffolds implanted post-auricularly, more realistic than on animal dorsum	• Animal study • 8 week end point • Study size unknown • Lack of comparison of neocartilage to native cartilage • Poor description of in vivo application • No testing for cartilage calcification or bone formation	• Separate studies for in vitro and in vivo application • Longer duration allowing for shape and size changes • Implant removal and QPCR analysis for bone markers along with alizarin staining to rule out calcification
Bichara et al. (2014) (60)	• Neocartilage identified throughout cross section of construct with even distribution • Optimal ovine neocartilage formation following 2 weeks in vitro culture • Neocartilage quality improved with increased implantation time • Ovine elastin detected at 12 weeks in vivo • No scaffold extrusion, localised swelling or erythema	• Neocartilage quality reduced with increased in vitro culture duration • Ovine neocartilage showed significantly reduced GAG compared to native cartilage • Some shrinkage of construct at 6 weeks in vitro	• Clear description of scaffold design and in vitro methods • Histological analysis and staining of auricles • Prospective study • Ethical approval	• Animal study • 12 week end point • Scaffold implanted subcutaneously in neck and subject to less pressure than post-auricular placement • No testing for cartilage calcification or bone formation	• Longer follow up allowing for shape and size changes • Implant removal and QPCR analysis for bone markers along with alizarin staining to rule out calcification
Sterodimas et al. (2013) (61)	• Ears maintained shape and flexibility • Histological analysis showed evidence of cartilage formation, type II collagen and matrix • No extrusion or infection of implants	• All showed reduction in both pinna height and width after 8 weeks	• Animals treated as per international guidance • Prospective • Clear methods • Primary outcomes of shape, size and histology addressed	• Animal study • 6 subjects • 8 week end point • Pinna flexibility measured subjectively using forceps • Aesthetics not considered	• Larger sample • Longer duration allowing for shape and size changes • QPCR analysis following implant removal for bone markers along with alizarin staining to rule out calcification

Yanaga et al. (2009) (21)	• First successful human implantation of regenerated cartilage tissue • Neocartilage showed adequate strength and elasticity for auricle reconstruction • No evidence of neocartilage absorption over 2-5 year follow up • Immunohistochemistry showed evidence of type II collagen formation	• Variable surgical techniques for shaping cartilage	• Ethical approval • Prospective • Clear methods • Microtia cartilage used hence minimal donor site morbidity • Reduced chance of rejection given autologous cartilage	• 4 subjects • 50% of subjects underweight, potential impact on wound healing • 2-5 years follow up not allowing fully for reabsorption • Neocartilage required sculpting by hand therefore range of techniques and outcomes • Two-stage implementation hence multiple surgeries	• Larger sample of patients • One surgeon for all cartilage reconstruction surgeries • QPCR analysis for bone markers along with alizarin staining to rule out calcification as quality control pre-implantation • MRI/CT scan to identify possible calcification of implant once in-situ

Table III: Critical Analysis of Literature

Abbreviations: QPCR quantitative polymerase chain reaction, PGA polyglycolic acid; PCL polycaprolactone; SEM scanning electron microscopy; 3D 3 dimensional; MRI magnetic resonance imaging; CT computed tomography; GAG glycosaminoglycan; bFGF (FGF-2) basic fibroblast growth factor; CAD computer aided design;